COMMISSION OF THE EUROPEAN COMMUNITIES

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EURATOM

PROGRESS REPORT

PROGRAMME

RADIATION PROTECTION

1980

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for the Commission of the European Communities

Progress Report

Programme 1976-1980

RADIATION PROTECTION

1980

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COMMISSION OF THE EUROPEAN COMMUNITIES
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EURATOM

Beretning
Program 1976-1980

STRÅLINGSBESKYTTELSE

Tätigkeitsbericht
Programm 1976-1980

STRAHLENSCHUTZ

Progress Report
Programme 1976-1980

RADIATION PROTECTION

Rapport d'Activité
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RADIOPROTEZIONE

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** Biology Group of the Commission of the European Communities DG XII, Biology, Radiation Protection and Medical Research, at the Ispra Establishment of the Joint Research Centre.

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I

EINLEITUNG

INTRODUCTION

INTRODUCTION

I. EINLEITUNG

Einer der im Vertrag zur Gründung der Europäischen Atomgemeinschaft vorgesehenen Forschungsbereiche umfasst Untersuchungen über schädliche Strahlenwirkungen auf Lebewesen, über die Verhütung und den angemessenen Schutz sowie die entsprechenden Sicherheitsnormen, den Nachweis und die Messung der Strahlungen sowie die Entwicklung therapeutischer Massnahmen gegen Strahlenwirkungen.

Seit 1960 sind unter dem Patronat der Kommission vier mehrjährige Forschungsprogramme auf dem Gebiet des Strahlenschutzes durchgeführt worden. Das Programm, das 1980 abgeschlossen wurde, kann wegen seines integrierten Charakters, der Aktualität der behandelten Themen, der praktischen Bedeutung der erzielten Fortschritte und Ergebnisse und der Eingliederung der Arbeiten in einen echten Gemeinschaftsrahmen als besonders wichtig angesehen werden.

Deshalb hat die Kommission in dem vorliegenden Bericht die im Zeitraum 1976/1980 erzielten Ergebnisse der Forschungsarbeiten zusammengestellt. Der Bericht erlaubt im Einzelfall die Tendenzen der Forschung, ihre Orientierung und ihre Prioritäten zu erkennen. Es handelt sich, wie bei den früheren Programmen, um ein Programm der indirekten Aktion, das im Rahmen von Verträgen mit Institutionen der Mitgliedstaaten, wie nationalen Forschungszentren und Hochschulen, durchgeführt wurde; der Etat betrug 39 MioECU für den Referenzzeitraum.

Es wird allgemein anerkannt, dass die von der Kommission herbeigeführte Zusammenarbeit in der Gemeinschaft auf dem Gebiet des Strahlenschutzes exemplarisch ist, wofür sowohl der Umfang und der Wert der wissenschaftlichen Veröffentlichungen (über 600 jährlich) als auch die Zahl der unmittelbar an den Forschungsverträgen mitwirkenden Wissenschaftler (über 500) sprechen. Die engen Beziehungen zwischen den Dienststellen der Kommission und den Forschern zeigen sich in immer stärkerem Masse in der gemeinsamen Arbeit in den Studiengruppen. Die Kommission hat jedes Jahr rund 40 solcher Sitzungen veranstaltet, die ausser Studiengruppen auch noch Konferenzen und Symposien umfassten; über 1.000 Wissenschaftler aus den Mitgliedstaaten und aus Drittländern nahmen daran teil. Diese Koordinierungsmassnahmen haben auf indirekte Weise einen beachtlichen Einfluss auf fast die gesamte Strahlenschutzforschung in der Gemeinschaft ausgeübt.

Auf dem Gebiet des Strahlenschutzes und der Strahlenbiologie gibt es tatsächlich eine europäische Wissenschaftsgemeinschaft, in der die Kommission Impulse gibt und Fördermassnahmen trifft, deren Notwendigkeit und Effizienz unbestritten sind. Aus der Gesamtheit der Arbeiten dieser Gemeinschaft bezieht die Kommission ferner die unerlässliche Unterstützung bei ihrer im Vertrag vorgesehenen normativen Aktion, in deren Rahmen sie 1959 erstmalig europäische Strahlenschutznormen festgelegt und damit eine gemeinsame Gesundheitspolitik eingeleitet hat. Diese Normen, die sich weitgehend auf internationale Empfehlungen stützen, werden durch die europäische Forschung untermauert und gegebenenfalls auf praktischer Ebene durch biologische und technologische Daten aus dem Programm vervollständigt.

Während die Forschungsaktionen der sechziger Jahre noch punktuellen Charakter hatten, kam es in der Folgezeit zu einer zunehmenden Kohärenz der verschiedenen Programmthemen und zu einer engen Zusammenarbeit zwischen Instituten mit ähnlichen Forschungsaufgaben. Diese Integration kennzeichnet das Fünfjahres-programme 1976-1980. Auf ihr bauen die Leitlinien des 1981 angelaufenen neuen Programms auf und gewährleisten damit die Kontinuität der Arbeiten und ihrer sozialen und humanen Motivierung.

Obwohl die Nutzung des Atoms bei der Öffentlichkeit weiterhin auf Skepsis und bisweilen übertriebene Befürchtungen stösst, hat die Energiekrise von 1973 einige europäische Länder bewogen, verstärkt die Kernenergie zur Elektrizitätserzeugung heranzuziehen. Die Befürchtungen ergeben sich zum grossen Teil aus Zweifeln und aus Lücken in der tatsächlichen Kenntnis der Langzeitwirkungen ionisierender Strahlungen bei schwacher Dosis. Diese Lücken zu füllen ist Zukunftsaufgabe der Strahlenschutzforschung.

Die ionisierenden Strahlungen sind von allen durch den Menschen verursachten Umweltbelastungen wahrscheinlich diejenigen, die am besten bekannt sind und am eingehendsten untersucht werden, dessen ungeachtet muss sich die Abschätzung des Strahlenrisikos auf möglichst genaue Beziehungen zwischen einer bestimmten Strahlenexposition und ihrer Auswirkung auf die Bevölkerung abstützen lassen. Mit den vorhandenen Modellen konnte das Risiko anhand von wissenschaftlichen Hypothesen beurteilt werden, die beim gegenwärtigen Stand der Kenntnisse durch epidemiologische Untersuchungen und Experimente

nachgeprüft werden. Darum müssen für erfolgversprechende epidemiologische Untersuchungen langfristig personelle und finanzielle Mittel eingesetzt werden.

Das Ziel des Forschungsprogramms "Strahlenschutz" ist es, das Mass an Gewissheit und an Sicherheit zu erhöhen. Die Bemühungen, die bisher unternommen wurden, um Klarheit über die Strahlenwirkungen zu erlangen, müssen mit Ausdauer und Ideenreichtum fortgesetzt werden, um Ergebnisse zu gewinnen, die weniger zweideutig und weniger widersprüchlich sind.

Zu den Grundsätzen des Strahlenschutzes gehört die Notwendigkeit der Optimierung, d.h. die Suche nach einem Gleichgewicht zwischen den Vor- und Nachteilen einer nuklearen Tätigkeit. Die Optimierung ist nur möglich, wenn der Strahlenschaden bewertet werden kann; diese Bewertung muss sich auf ein vergleichsweise komplexes, multidisziplinäres wissenschaftliches Verfahren stützen, für das im Rahmen des soeben abgeschlossenen Programms die ersten Entwicklungsschritte getan werden konnten. Von den bereits vorliegenden Ergebnissen ausgehend, sieht das neue Forschungsprogramm für diesen Bereich einen nennenswerten Ausbau vor.

Die kanzerogene Wirkung der ionisierenden Strahlungen steht im Vordergrund der Besorgnisse. Die in diesem Bericht behandelten Arbeiten lassen die eingeschlagenen Wege erkennen, um den Mechanismus der induzierten Mutagenese und der Karzinogenese zu klären. Eine solche Untersuchung erfordert epidemiologische Forschungen, tierexperimentelle Forschungen sowie Untersuchungen im zellularen und molekularen Bereich. Wenn diese verschiedenen Ansätze Gegenstand systematischer und koordinierter Bemühungen sind, wird es eines Tages wahrscheinlich möglich sein, die noch offenen Fragen hinsichtlich der strahleninduzierten Karzinogenese und der Abschätzung des Krebsrisikos zu zu beantworten. Die Virologie und die Immunologie werden dazu einen wichtigen Beitrag leisten. Bis dahin bleiben noch zahlreiche Lücken zu schliessen bei so wichtigen Faktoren wie den molekularen Vorgängen der Reparation strahleninduzierter Schäden, der immunologischen Reaktion und der Wechselwirkung zwischen Krebszellen und dem Immunsystem.

Die Bestrahlungen zu medizinischen Zwecken haben den grössten Anteil an der künstlichen Strahlenbelastung der Bevölkerung. Da diese Bestrahlungen in den Industriestaaten ständig zunehmen, muss unbedingt untersucht werden, wie sie - allerdings ohne jede Beeinträchtigung der Diagnostik - begrenzt werden können. Dieses Problem wird im Rahmen des folgenden Programms zweifellos verstärkte Aufmerksamkeit finden.

Ein vollständiger Überblick über die Ergebnisse kann in dieser kurzen Einleitung nicht gegeben werden. Die allgemeinen Leistungen der Kommission auf dem Gebiet des Strahlenschutzes lassen sich schematisch etwa folgendermassen zusammenfassen:

- Entwicklung langfristiger Forschungsprogramme,
- Intensivierung der Forschung auf Gebieten mit besonderer Wichtigkeit für die Öffentlichkeit,
- Förderung der Übertragung wissenschaftlicher Ergebnisse in die praktische Anwendung, z.B. Entwicklung von Personendosimetern, Diagnose von akuten Strahlenschäden und ihre Behandlung, (z.B. durch Knochenmarktransplantation), Durchführung von Vergleichsprogrammen,
- Aufstellung langfristiger Prognosen, z.B. hinsichtlich der Toxizität von Tritium für den Organismus,
- Förderung der Einbeziehung wissenschaftlicher Erkenntnisse in politische Entscheidungsprozesse, z.B. Erstellung von Strahlenschutz-Grundnormen, Erarbeitung von Kriterien für die Standortwahl, vergleichende Untersuchung der Risiken, die mit den verschiedenen Energieerzeugungsanlagen verbunden sind.

Wir denken, dass diese Veröffentlichung eine Vorstellung von dem Umfang der einzelstaatlichen und der gemeinschaftlichen Bemühungen zur Förderung der Forschung im Bereich Strahlenschutz vermittelt. Diese Forschung ist bestrebt, die Forderungen des Strahlenschutzes zu erfüllen; sie steht nach wie vor in enger Verbindung mit der normativen Tätigkeit der EG-Kommission. Sie ist auf die globale Erfassung des Radioaktivitätsrisikos ausgerichtet, denn sie beschäftigt sich mit der Gesamtheit der schädlichen Wirkungen und trägt zur Entwicklung der Grundsätze für die Optimierung der nuklearen Tätigkeiten bei.

F. VAN HOECK P. RECHT

I. INTRODUCTION

One of the research areas covered by the Treaty establishing the European Atomic Energy Community is the study of the harmful effects of radiation on living beings as well as of adequate prevention and protection measures and corresponding safety standards, radiation detection and measurement and therapy to counteract the effects of radiation.

Four multiannual research programmes relating to radiation protection have been implemented since 1960 under the auspices of the Commission; the programme which was completed in 1980 seems particularly important because of its integrated nature, the topicality of the subjects studied, the practical importance of the progress achieved, of the results obtained, and the integration of the work into a genuine Community effort.

This is why the Commission has grouped together in these volumes the results of the work conducted by the contractors during the period 1976-80 and presented in a general overview which gives a clearer picture of the trends in research and the patterns followed by policies and priorities in each field. As in the case of preceding programmes, the present one consists in indirect action implemented through contracts with organizations in the Member States such as national research centres and universities; it was allocated a budget of 39 million ECU for the period under consideration.

It is generally recognized that the Community cooperation established by the Commission in the field of radiation protection is exemplary both in terms of the importance and value of the scientific publications (in excess of some 600 per year) and because of the number of researchers (nearly 500) directly involved in the research contracts. Furthermore, the existence of special lines of communication between the researchers and the Commission departments is becoming increasingly evident in the study groups. The Commission has held on average some 40 meetings each year, which have not been merely study groups but have also taken the form of conferences and symposia; such meetings have been attended by more than a thousand scientists from Member States and non-member countries. These coordination activities exert a considerable indirect influence on virtually all research undertaken on radiation protection in the Community.

There exists today in the radiation protection and radiobiology field a genuine European scientific community within which the Commission plays a stimulation and promotion role whose need and effectiveness are beyond question. The body of work conducted by this community also provides the essential scientific support for the legislative activities that the Commission is developing in accordance with the Treaty and in pursuance of which it drew up for the first time in 1959 the Community's Basic Safety Standards for radiation protection, which constituted the initiation of a common health policy.

These standards, which derive to a considerable extent from international recommendations, are thus confirmed by European research and supplemented if necessary in practical terms by the biological and technological data that result from the programme.

The research projects carried out during the sixties were of a fragmentary nature, which gradually gave way to a greater coherence between the various topics in the programme and the establishment of closer collaboration between institutions pursuing similar research objectives. This more advanced integration is a salient feature of the 1976-80 programme; it has greatly influenced the policy lines incorporated in the new programme which started in 1981 and has provided continuity in the conduct of the research and ensured the stability of its social and human motivations.

While the use of the atom continues to arouse considerable anxiety and often exaggerated fears among the general public, the 1973 energy crisis prompted a number of European countries to opt for nuclear energy as a means of generating electricity. Many of these fears are based on doubt and insufficient actual knowledge of the seriousness of the long-term effects of ionizing radiation in low doses. In order to provide the answers to these questions, radiation protection research has to be further developed.

Although ionizing radiation is doubtless the best known and most thoroughly researched of all the different types of pollution caused by man, the risks must nevertheless be quantified on the basis of as accurate as possible relationships between exposure at a given level and the probability of the occurrence of an effect among the general public. Currently available models have made it possible to perform risk evaluation by adopting scientific assumptions that in the present state of knowledge are checked through epidemiological investigation and experiment.

Epidemiological studies in particular require considerable long-term investment in terms of both staff and financial resources. The aim of any research programme is to provide even greater certainty and safety. It is essential that the efforts made hitherto in order to ascertain the effects of radiation be continued with the patience and imagination that are needed for the results to be clearer and les contradictory.

One of the principles underlying radiation protection is the need to optimize, in other words, to attempt to strike a balance between the advantages and the disadvantages of a nuclear activity. Optimization is only possible if an evaluation of radiological damage can be carried out, and this evaluation must be based on a relatively complex, multi-disciplinary scientific activity whose initial developments have been rendered possible by the programme that has just been completed. Building on the results that have already been obtained, the new programme has considerably enlarged this area.

The risk that give the greatest grounds for anxiety include the carcinogenic effect of ionizing radiation. The work described in this publication shows inter alia how knowledge of the process of induced mutagenicity and carcinogenicity has been envisaged. This study combines epidemiological research, experimental research conducted on animals and cellular and molecular research. If a systematic, coordinated effort is made to advance along these different lines of approach, it is probable that one day answers will be found to the remaining unknowns in the field of radiation-induced carcinogenicity and the estimation of cancer risk. Virology and immunology also provide significant contribution to the study of this problem. Nevertheless, many gaps remain in our knowledge of questions as fundamental as the molecular processes whereby radiation-induced damage is repaired, and whereby immunological response and the interaction between cancerous cells and the immunizing system are governed.

Irradiation for medical purposes is the largest source of man-made irradiation of the general public, moreover, this form of irradiation is steadily increasing in the industrialized countries and it is extremely important to investigate methods of reducing it while in no way hindering diagnosis. The effort devoted to this problem will without doubt be intensified during the forthcoming programme.

It is not possible in this short introduction to give an exhaustive summary of the results. The Commission's general achievements in the field of radiation protection can, however, be outlined as follows :

- the preparation of long-term research projects;
- the intensification of research activities in fields that are of crucial importance to the general public;
- the promotion of practical applications of the results of scientific research for example, the development of personal dosimeters, the diagnosis and treatment (in particular by means of bone-marrow transplants) of major radiation-induced lesions, and the implementation of intercomparison programmes;
- the formulation of longer-term forecasts for example, as regards the toxicity of tritium on the body;
- assistance in the integration of the results of scientific research into the political decision-making process for example, the establishment of Basic Safety Standards in the radiation protection field, the drafting of criteria for the siting of nuclear plants; and the compilation of a comparative study of the hazards from the various energy-producing industries.

We therefore consider that this publication will demonstrate the importance of the work performed by the Member States and the Community in promoting research in the radiation protection field. This research is attempting to provide solutions to the essential problems met by radiation protection, and it remains closely linked with the European Commission's legislative activity. It sets out to adopt an overall approach towards the radioactive hazard, considering all damaging effects and contributing to the development of the principles on which nuclear activities are optimized.

F. VAN HOECK. P. RECHT.

I. INTRODUCTION

Un des domaines de recherche prévus par le Traité instituant la Communauté européenne de l'Energie atomique est l'étude des effets nocifs des radiations sur les êtres vivants, de la prévention et de la protection adéquates et des normes de sécurité correspondantes, la détection et la mesure des radiations, ainsi que l'étude de la thérapeutique contre les effets des radiations.

Quatre programmes de recherches multiannuels en matière de radioprotection ont été mis en oeuvre depuis 1960, sous l'égide de la Commission ; celui qui s'est achevé en 1980 apparaît comme particulièrement important en raison de son caractère intégré, de l'actualité des sujets traités, de la signification pratique des progrès réalisés et des résultats obtenus et de l'intégration des travaux en un véritable effort communautaire.

C'est pourquoi la Commission a regroupé dans les présents volumes les résultats des travaux des contractants obtenus pendant la période 1976/1980 et présentés dans une revue d'ensemble qui permet de mieux percevoir, pour chacun d'eux, les tendances de la recherche et l'évolution des orientations et des priorités. Il s'agit, comme pour les programmes précédents, d'un programme d'actions indirectes exécuté par voie de contrats avec des organismes des Etats membres, tels que centres nationaux de recherche et universités ; le budget afférent à cette action étant de 39 MioECU pour la période considérée.

Il est généralement reconnu qu'en radioprotection la coopération communautaire établie par la Commission apparaît comme exemplaire, et cela tant par l'importance et la valeur des publications scientifiques (plus de 600 environ par année), que par le nombre de chercheurs directement impliqués (près de 500) dans les contrats de recherche. Par ailleurs, l'existence de liens privilégiés entre les chercheurs et les services de la Commission se manifeste de façon sans cesse accrue dans les groupes d'études. En moyenne, la Commission a organisé chaque année une quarantaine de réunions qui ne se sont pas limitées à ces seuls groupes d'études mais ont également pris la forme de conférences et de symposia ; plus d'un millier de scientifiques provenant des Etats membres et de pays tiers ont participé à ces réunions. Ces travaux de coordination ont une influence indirecte considérable sur la quasi totalité des recherches en radioprotection entreprises dans la Communauté.

Il existe actuellement en radioprotection et radiobiologie une véritable communauté scientifique européenne au sein de laquelle la Commission joue un rôle d'animation et de promotion dont la nécessité et l'efficacité sont incontestables. L'ensemble des travaux de cette communauté constitue en outre le support scientifique indispensable à l'action normative que la Commission développe par ailleurs, conformément au traité, et qui l'a conduite à établir en 1959 pour la première fois les normes européennes de radioprotection, amorce d'une politique sanitaire commune. Ces normes, qui découlent largement de recommandations internationales, sont ainsi affermies par la recherche européenne et complétées, s'il y a lieu, sur le plan pratique, par des données biologiques et technologiques émanant du programme.

Les actions de recherche des années 60 avaient un caractère ponctuel qui a progressivement fait place à une meilleure cohérence entre les différents thèmes du programme et à l'instauration d'une collaboration plus étroite entre des instituts poursuivant des objectifs de recherche analogues. Cette intégration plus poussée caractérise le programme quinquennal 1976-1980 ; elle a fortement influencé les lignes directrices du nouveau programme qui a débuté en 1981 et assuré une continuité dans la conduite des études et la permanence de leurs inspirations sociales et humaines.

Alors que l'usage de l'atome continue à susciter dans l'opinion publique une appréhension certaine et des craintes souvent exagérées, la crise de l'énergie de 1973 a conduit un certain nombre de pays européens à choisir l'énergie nucléaire pour la production d'électricité. Une grande partie des craintes repose sur les doutes et les lacunes dans la connaissance réelle de la gravité des effets des radiations ionisantes, à faible dose et à long terme. Pour répondre à ces questions, la recherche en radioprotection doit encore se développer.

Certes, de toutes les pollutions d'origine humaine, les radiations ionisantes sont vraisemblablement les mieux connues et les plus étudiées. Il n'en demeure pas moins que la quantification des risques doit être fondée sur les relations les plus précises possible entre l'exposition à un niveau déterminé et la probabilité d'apparition d'un effet dans la population. Les modèles qui existent actuellement ont permis une évaluation du risque en adoptant des hypothèses scientifiques qui, dans l'état actuel des connaissances, sont vérifiées par l'enquête épidémiologique et par l'expérimentation.

Les études épidémiologiques notamment demandent un important investissement à long terme, en moyens humains et financiers. L'objectif de tout programme de recherche est d'assurer davantage de certitude et de sécurité. Il est essentiel que les efforts entrepris jusqu'à présent pour connaître les effets soient poursuivis avec la patience et l'imagination nécessaires pour que les résultats comportent moins d'ambiguité et moins de contradiction.

Un des principes de la radioprotection est la nécessité de l'optimisation, c'est-à-dire de la recherche d'un équilibre entre les avantages et les désavantage d'une activité nucléaire. L'optimisation n'est réalisable que s'il est possible d'effectuer une évaluation du dommage radiologique ; cette dernière doit reposer sur une démarche scientifique relativement complexe à caractère multidisciplinaire et dont le programme qui vient de s'achever a permis les premiers développements. C'est en partant des résultats déjà obtenus que le nouveau programme de recherche a donné à ce chapitre une particulière extension.

Parmi les effets dommageables les plus préoccupants figure l'action cancérogène des radiations ionisantes. Les travaux repris dans la présente publication permettent notamment d'apprécier de quelle manière a été envisagée la connaissance du mécanisme de la mutagenèse et de la carcinogénèse induites. Cette étude sollicite à la fois des recherches épidémiologiques, des recherches expérimentales effectuées chez l'animal et des recherches au niveau cellulaire et moléculaire. Si ces différentes approches font l'objet d'un effort systématique et coordonné, il est probable qu'un jour il sera possible de fournir une réponse aux inconnues qui subsistent toujours en ce qui concerne la carcinogénèse radioinduite et l'estimation du risque du cancer. La virologie et l'immunologie apportent également une contribution importante à l'étude de ce problème. Mais il subsiste encore de nombreuses lacunes dans la connaissance de questions aussi essentielles que le mécanisme moléculaire de la réparation du dommage radioinduit, de la réponse immunologique et de l'interaction entre les cellules cancéreuses et le système immunitaire.

Les irradiations d'origine médicale constituent la source la plus importante d'irradiation artificielle des populations. De plus, dans les pays industrialisés, ces irradiations sont en augmentation constante et il est très important d'étudier les moyens de les réduire en n'apportant toutefois aucune entrave au diagnostic. L'attention accordée à ce problème sera sans aucun doute intensifiée au cours du prochain programme.

Il n'est pas possible dans cette brève introduction de présenter une synthèse complète des résultats. Les réalisations générales de la Commission en matière de radioprotection peuvent être schématisées de la manière suivante :

- élaboration de projets de recherche à long terme,
- intensification de la recherche dans des domaines particulièrement importants pour la population,
- promotion des applications pratiques des résultats de la recherche scientifique (par exemple, mise au point de dosimètres individuels, diagnostic et traitement de lésions radioinduites graves, notamment par transplantation de moelle osseuse, exécution de programmes d'intercomparaison),
- établissement de pronostics à plus long terme (par exemple en ce qui concerne la toxicité du tritium pour l'organisme),
- aide à l'intégration des résultats de la recherche scientifique dans le processus de décision politique (par exemple, établissement de normes de base dans le domaine de la protection radiologique, élaboration de critères de choix de sites d'implantation, étude comparative des risques émanant des diverses industries productrices d'énergie).

Nous pensons donc que la présente publication permettra de juger de l'importance des efforts nationaux et communautaires dans la promotion de la recherche en radioprotection. Cette recherche tente de répondre aux impératifs de la protection radiologique ; elle reste étroitement liée à l'activité normative de la Commission européenne. Elle s'est orientée vers l'approche globale du risque radioactif, en envisageant l'ensemble des effets dommageables et en contribuant au développement des principes d'optimisation des activités nucléaires.

F. VAN HOECK P. RECHT

II

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Members in 1980 of the Advisory Committee on Programme Management

"BIOLOGY - HEALTH PROTECTION"

Membres en 1980 du Comité Consultatif en matière de Gestion de Programme

"BIOLOGIE - PROTECTION SANITAIRE"

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III

FORSCHUNGSTÄTIGKEIT STRAHLENSCHUTZ

RESEARCH IN RADIATION PROTECTION

RECHERCHES EN RADIOPROTECTION

III. 1.

STRAHLENMESSUNGEN UND IHRE INTERPRETATION (DOSIMETRIE)

MEASUREMENT AND INTERPRETATION OF RADIATION (DOSIMETRY)

MESURE DES RAYONNEMENTS ET LEUR INTERPRETATION (DOSIMETRIE)

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Tätigkeitsbericht beschrieben : *

Further research work on these subjects will also be described in the following progress reports : *

D'autres travaux sur ce thème de recherche sont également decrits dans les rapports suivants : *

185-BIA N ITAL, Wageningen (de Zeeuw/Ringoet)
167-BIO UK AERE, Harwell (Peirson)
205-BIO D GSF, Frankfurt (Pohlit)
201-BIO C EULEP, (Duplan et al.)
182-BIO UK NRPB, Harwell (Dennis/Smith)
243-BIO UK PCL, London (Simmons)
249-BIO UK MRC, Harwell (Vennart)
216-BIO D Univ. Erlangen (Pauly)
244-BIO D GSF, Neuherberg (Drexler)
245-BIO UK AERE, Harwell (Peirson).

* Siehe auch Punkt IV,
See also section IV,
Voir aussi point IV,

Contractor	: Radiobiological Institute TNO, Rijswijk, The Netherlands
Contract no.	: 199-76-1 BIO N
Head of Research Team	: G.W. Barendsen and J.J. Broerse
General subject of contract	: Evaluation of the biological effectiveness of various types of radiation for different types of damage in mammalian cells and measurements of absorbed dose, dose distribution patterns and radiation quality for energy deposition by fast neutron beams.

Project no. 1.

G.W. Barendsen, J.J. Broerse and J. Zoetelief

Measurements of the biological effectiveness of fast neutrons of different energies for cell reproductive death and for chromosome aberrations in different types of cultured mammalian cells.

The relative biological effectiveness of fast neutrons with different energies has been studied for the induction of cell reproductive death and of chromosome aberrations in different types of cultured mammalian cells. The aim of these studies is to obtain insight in the variability of RBE values for these endpoints among different types of cells and the correlation of the dependence of this RBE on neutron energy. It is further of interest to compare these values with the RBE for tumour induction obtained in other investigations with the same neutron energies. From a review of data in the literature it could be shown that RBE values for chromosome aberrations exhibit a stronger dependence on neutron energy than corresponding values for induction of cell reproductive death, especially at low radiation doses.

In a first series of experiments for a large number of types of exponentially growing mammalian cells, survival curves were measured for irradiations with fast neutrons with energies of 15 and 0.5 MeV and with 300 kV X rays. The absolute sensitivity of the cell lines investigated varied by a factor of 4 for X-rays and by a factor of 3 for 15 MeV neutrons. RBE values derived from these experiments showed that a wide range is obtained namely between 1.8 and 3.3 for 15 MeV neutrons and between 4.8 and 9 for 0.5 MeV neutrons.

In a second series of experiments the induction of chromosome aberrations has been studied in different types of cultured mammalian cells, namely

R-1,M, RUC-2 and V-79. The percentages of dicentrics and centric rings have been measured as a function of total absorbed dose for ^{137}Cs gamma rays, 300 kV X rays and fast neutrons with energies of 0.5, 4.2 and 15 MeV. The RBE values derived at two levels of induced chromosome aberrations are shown in Table 1. Also given in this table are the doses of X rays necessary for the induction of 10 and 30 percent of aberrations in the different cell lines, which indicate the differences in absolute sensitivity of the different cell lines.

For the studies on induction of chromosome aberrations plateau phase cell cultures have been used, since these cultures have a large fraction of cells in the G_1 phase of the cell cycle. Therefore, studies on cell reproductive death had also to be carried out for these types of cultures. The preliminary RBE values of various types of radiation at two levels of cell survival are given in Table 2 for the three cell lines employed. As a measure for the absolute sensitivity of the cells, the doses of X rays for 50 and 10 per cent survival are indicated. The following conclusions have been made.

The different cell lines show a considerable variation in sensitivity to induction of cell inactivation and chromosome aberrations. With regard to cell inactivation, R-1,M cells show the highest sensitivity to all types of radiation but, for induction of dicentrics and centric rings, V-79 cells show the highest susceptibility for irradiations with neutrons. The effectiveness for induction of both types of effect is highest for 0.5 MeV neutrons, intermediate for 4.2 and 15 MeV neutrons and lowest for photons. Differences in the effectiveness of 4.2 and 15 MeV neutrons for induction of both types of effect are observed for V-79 cells, but for R-1,M and RUC-2 cells 4.2 and 15 MeV neutrons show approximately equal effectiveness for both types of effect. Differences in effectiveness between ^{137}Cs gamma rays and 300 kV X rays were observed only for RUC-2 cells for both types of effect and for induction of chromosome aberrations in V-79 cells at dose levels in excess of about 3.5 Gy. At the same levels of effect for 300 kV X rays for all cell types, somewhat higher RBE values are observed for induction of cell reproductive death than for induction of dicentrics and centric rings. At 50 per cent survival, maximum RBE values of about 10 are found for V-79 and RUC-2 cells irradiated with 0.5 MeV neutrons. Dicentrics and centric rings can explain only about a fraction of 0.2 to 0.4 of the impairment of clonogenic capacity for the different cell lines. More extensive information has to be obtained to allow a more detailed analysis in terms of, e.g., linear-quadratic dose response relationships. Additional scoring of acentric fragments is of

interest. In future studies the cause for the different sensitivities of the different cell lines to ionizing radiation might be investigated with regard to differences in production or repair of initial damage, relevant for the effects of fractionated or protracted irradiations at low doses and low dose rates.

RBE VALUES OF VARIOUS RADIATIONS AT TWO LEVELS OF INDUCED CHROMOSOME ABERRATIONS FOR THREE CELL LINES

Type of rafiation	R-1,M cells RBE at 10% of aberrations	RUC-2 cells RBE at 10% of aberrations	RUC-2 cells RBE at 30% of aberrations	V-79 cells RBE at 10% of aberrations	V-79 cells RBE at 30% of aberrations
^{137}Cs γ-rays	0.9 ± 0.2	0.7 ± 0.2	0.7 ± 0.2	1.1 ± 0.3	0.9 ± 0.2
15 MeV neutrons	1.9 ± 0.4	2.9 ± 0.7	2.4 ± 0.4	3.0 ± 0.7	1.8 ± 0.4
4.2 MeV neutrons	1.9 ± 0.4	2.6 ± 0.7	-	4.3 ± 0.9	3.3 ± 0.6
0.5 MeV neutrons	4.4 ± 0.8	5.0 ± 1.3	3.7 ± 0.7	7.0 ± 1.4	5.1 ± 0.9
corresponding dose levels of 300 kV X-rays	1.6 Gy	2.7 Gy	6.0 Gy	2.6 Gy	5.2 Gy

RBE VALUES OF VARIOUS RADIATIONS AT TWO LEVELS OF CELL SURVIVAL FOR THREE CELL LINES

Type of radiation	R-1,M cells RBE at 50% survival	R-1,M cells RBE at 10% survival	RUC-2 cells RBE at 50% survival	RUC-2 cells RBE at 10% survival	V-79 cells RBE at 50% survival	V-79 cells RBE at 10% survival
^{137}Cs γ-rays	1.0 ± 0.2	1.0 ± 0.2	0.8 ± 0.2	0.8 ± 0.2	1.0 ± 0.2	1.0 ± 0.2
15 MeV neutrons	2.0 ± 0.4	1.7 ± 0.4	3.0 ± 0.5	1.7 ± 0.3	2.8 ± 0.4	2.0 ± 0.3
4.2 MeV neutrons	-	-	-	-	4.8 ± 0.9	2.8 ± 0.6
0.5 MeV neutrons	6 ± 1	4.4 ± 0.9	9 ± 2	5.1 ± 0.9	12 ± 2	5.3 ± 0.9
corresponding dose level of 300 kV X-rays	2.1 Gy	5.3 Gy	5.0 Gy	8.6 Gy	4.9 Gy	9.0 Gy

Project no. 2.

J. Zoetelief and J.J. Broerse

Measurements of absorbed dose and radiation quality inside a human phantom.

New regulations in radiation protection necessitate the knowledge of organ doses for relevant standardized exposure conditions. The dose distribution in anthropomorphic phantoms has been determined with a number of different detectors including tissue equivalent (TE) and Mg-Ar ionization chambers, pulse fission counters and Geiger-Müller counters. For measurements with TE ionization chambers, it is further of importance to ascertain the effective point of measurement for neutron beams of different energies.

When ionization chambers are used under free-in-air conditions, the geometrical centre of the chamber is the effective point of measurement, if the distance to the source is in excess of five times the diameter of the chamber. For measurements in a phantom, the effective point of measurement can be displaced from the geometrical centre due to the replacement of phantom material by the gas filled cavity of the ion chamber. A correction has to be applied for changes in attenuation and scattering of the radiation under these different conditions.

Three spherical tissue-equivalent (TE) ion chambers with internal diameters of 8, 16 and 32 mm and wall thickness of 2.2 mm were used. The central electrodes consisted of a TE sphere (radius 2 mm) on a TE stem (radius 1 mm). The TE chambers were flushed with TE gas; all measurements were performed at both polarities of the ionizing potential and corrected for incomplete ion collection and leakage current.

The studies on effective measuring point have been performed with X rays, ^{137}Cs and ^{60}Co gamma-rays, fission neutrons, and neutrons produced by the d+D, d+T and d+Be reactions. For the high energy neutron and photon irradiations differences in absorbed dose were observed for the different spherical chambers at the same depth of measurement in the water phantom. These differences were converted by using the depth dose curves to differences in the effective point of measurement. When the displacement of the effective point of measurement from the chamber centre was plotted against the chamber radius, a linear relationship resulted. The displacements of the effective point of measurement were therefore extrapolated to zero chamber radius and normalized to the value obtained as shown in Figure 1.

The linear relationship between the displacement of the effective point of measurement, d, and the chamber radius, r, can be presented as $d = (0.23 \pm 0.06)r$ and $d = (0.30 \pm 0.06)r$ for d+T neutrons at Rijswijk and Amsterdam, respectively, which are significantly smaller than the displacement for ^{60}Co gamma rays of $(0.58 \pm 0.06)r$. Displacement correction factors, δ, can be calculated as the ratio of the actual dose (for an infinitesimally small cavity) to the dose measured. For both d+T neutron beams, the same displacement correction factor of $1-(0.25 \pm 0.06).10^{-2}.r$ is observed when r is expressed in mm, which suggests that the correction factor is independent of SSD or field size. This finding indicates a serious drawback in the concept of radial displacement, which is dependent on SSD. Therefore, further evaluation of the results have been made in terms of displacement correction factors, δ.

A summary of δ values for photons and neutrons of different energies is given in Table 3. The displacement correction factor for spherical ion chambers in a water phantom for ^{60}Co gamma rays is smaller than that for ^{137}Cs gamma rays while, for X rays, no displacement is found. The derived displacement correction factors, δ, for in-phantom measurements show a decreased displacement with decreasing photon energy. For neutrons also a dependence of δ on neutron energy is observed. For relatively low energy neutrons, no displacement was found, whereas, for neutrons with energies in excess of 5.3 MeV, δ shows an almost constant value.

The correction for displacement in a neutron or photon field should preferably be made in terms of a correction factor which is dependent on the chamber dimensions and shape and neutron or photon energy and independent of depth in phantom, field size and SSD. This correction factor will most probably not vary much with phantom size and density of the phantom material. It should be realized that this factor should be applied only for depths in excess of that of the dose maximum. For most neutron and photon beams this will not lead to severe difficulties, since the dose maximum is generally located at depths less than 1 cm. The results indicate that the correction for displacement of the effective point of measurement is not a geometrical problem depending only on chamber shape, but results from the complex balance between differences in attenuation and scattering of the various radiation qualities caused by the introduction of a gas filled cavity into a phantom.

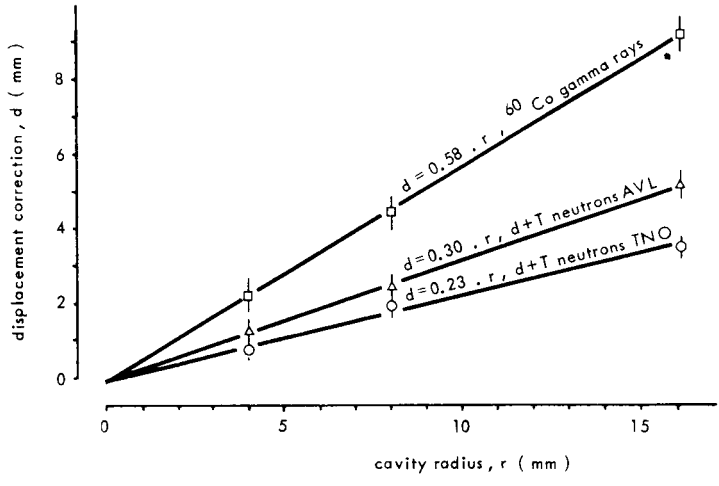

Figure 1.
Radial displacement, d, versus cavity radius, r, for spherical TE ion chambers.

DISPLACEMENT CORRECTION FACTORS, δ , OF SPHERICAL IONIZATION CHAMBERS FOR MEASUREMENTS IN PHANTOMS WITH DIFFERENT TYPES OF RADIATION

type of radiation	δ
150, 200 and 300 kV X-rays	$1.000 \pm 0.05.10^{-2}.r$
^{137}Cs γ rays	$1-(0.22 \pm 0.05).10^{-2}.r$
^{60}Co γ rays	$1-(0.37 \pm 0.04).10^{-2}.r$
fission neutrons ($\bar{E}_n$ = 1 MeV)	$1.000 \pm 0.1.10^{-2}.r$
d(2.3)+D neutrons (E_n = 5.3 MeV)	$1-(0.25 \pm 0.09).10^{-2}.r$
d(0.25)+T neutrons (E_n = 14.2 MeV)	$1-(0.25 \pm 0.06).10^{-2}.r$
d(0.5)+T neutrons (E_n = 14.8 MeV)	$1-(0.25 \pm 0.06).10^{-2}.r$
d(50)+Be neutrons ($\bar{E}_n$ = 21 MeV)	$1-(0.21 \pm 0.05).10^{-2}.r$

Project no. 3

J.J. Broerse, B. Hogeweg, B.J. Mijnheer and G.W. Barendsen

Determination of biologically effective dose at various positions in a human phantom.

Studies have been made to determine changes in RBE values of different types of radiation as a function of the location in the human body for a variety of exposure conditions. In many types of exposure, inhomogeneous irradiation of persons will occur whereby parts of the body receive doses of different quality due to scattering and absorption of the primary radiation. For the assessment of the distribution of the biologically effective dose a number of studies have been carried out concerning microdosimetric parameters and responses of biological systems.

Studies on the effectiveness for reproductive death of cells have been carried out for different positions inside a human phantom employing different types of radiation. Cell irradiations at different depths in a human phantom have been performed with 600 MeV alpha particles at Saclay (in cooperation with Drs. A. and J. Dutreix) and with fast neutrons produced by the d+T and the d+Be reaction. The latter experiments have been performed in cooperation with Dr. A. Wambersie with the neutron beam produced by the CSF isochronous cyclotron Cyclone at Louvain-la-Neuve.

Perturbations of charged particle equilibrium at interfaces of materials of different atomic compositions can lead to considerable differences in the energy deposition by photons and neutrons. Specific examples of these interface perturbations are encountered during irradiations of body cavities and soft tissue adjacent to or enclosed by bone, and irradiations of cells in monolayer on the bottom of culture dishes.

The survival curves of cultured T-1 cells of human origin were measured for 14.5 MeV neutron irradiation in two geometries whereby the incident neutrons passed through medium or through bottoms of the dishes. As indicated in Figure 2 for a given tissue kerma, a higher level of survival is observed for the irradiations through the polystyrene bottom of the flasks. This indicates that a smaller amount of energy is absorbed in the cells in this situation. The relative absorbed doses in tissue layers irradiated through water or through polystyrene differ by a factor of 1.16. Cell irradiations with 15 MeV neutrons are generally performed with the neutron beam reaching the cells through the medium. Under these conditions we account for the slight increase in neutron dose by applying a correction of 5 per cent relative to soft tissue. It should be realized, however, that large correction factors will be required for neutron irradiations through the bottom of the flasks.

To estimate the changes in radiation quality using microdosimetry, linear energy spectra were determined for positions in and outside collimated beams of 15, 6.5 and 0.51 MeV neutrons. The different energy neutrons were produced, respectively, by the d(.28)+T, d(3.5)+D and p(1.34)+T reactions and were collimated with an experimental arrangement at the Radiobiological Institute TNO. The size of the exit field, defined by the tapered steel insert, is 6 x 8 cm^2. The distributions of the lineal energy (y) were measured with a tissue-equivalent cylindrical proportional counter at three different positions behind the collimator: at the centre of the beam, at the geometrical edge (as defined by the insert) and behind the shielding at a distance of 4 cm from the longest boundary side (equivalent to 7 cm from the center).

The fractional dose distributions derived for center position and the geometrical edge were almost identical for the three neutron energies used. As a result of the neutron scattering on the inner duct, the distribution for a boundary showed only a slight increase of events with y values around 1000 MeV cm^{-1}. For positions outside the beam behind the shielding, the fractional dose distributions for 15 and 6.5 MeV neutrons showed increasing contributions of gamma rays and attenuated and scattered neutrons.

Changes in quality with position were also determined by a biological dosimetry method employing cell survival as the quantitative endpoint. Mammalian cells were irradiated in flasks and tubes at various positions in a cubical water phantom (side lengths 30 cm) with different doses of 6.5 and 15 MeV collimated neutrons. As a typical example, survival data derived for cells irradiated at different depths with 15 MeV neutrons are presented in Figure 3. Similar survival data have been derived for cells irradiated in positions perpendicular to the beam axis. From these results it can be concluded that the RBE did not vary significantly in and outside the beam region. For positions in the beam, this is in agreement with the small changes which were observed in the lineal energy spectra. The absence of a RBE change for positions behind the shield is in contrast with the large increase in events having high y values in the fractional dose distribution, but can be explained by the counterbalancing effects of the increasing contribution of gamma rays at these positions.

It can generally be concluded that microdosimetry provides a suitable basis for radiation quality specification but that a pragmatic approach has to be adopted. Possible differences in the radiation quality of different fast neutron beams can be assessed by microdosimetric techniques, but should be supported by comparison of the response of biological dosimeters in the different beams.

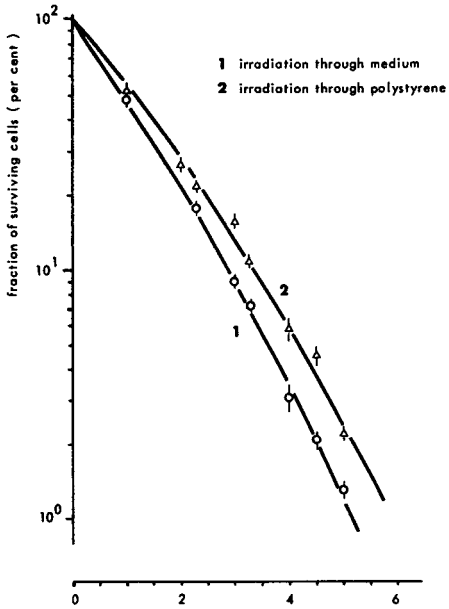

Figure 2.

Survival curves of cells irradiated with d+T neutrons through the medium or through the polystyrene bottom of the culture flasks.

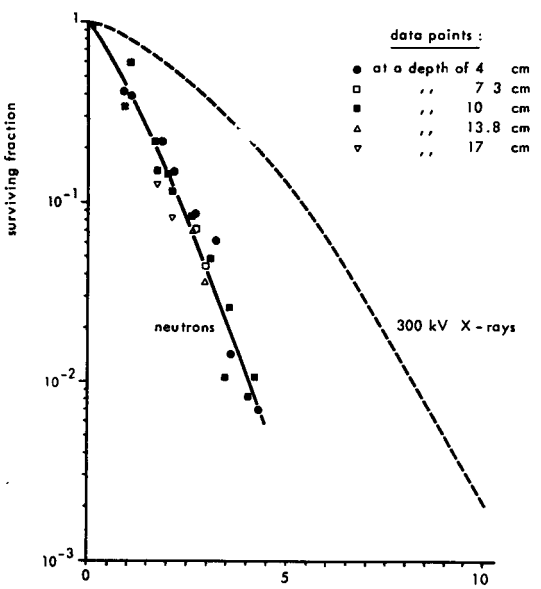

Figure 3.

Survival of cultured cells at different depths in a phantom irradiated with 15 MeV neutrons along the central axis of the beam.

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Contractant de la Commission : Association pour le Développement de la Physique Atomique
118 route de Narbonne
31062 TOULOUSE CEDEX

Centre de Physique Atomique
Université Paul Sabatier

N° du contrat : 176 76 1 BIO F

Chef du groupe de recherche : Professeur D. BLANC

Thème général du contrat : Transport simulation of electrons, photons and heavy particles by Monte-Carlo methods. Applications to microdosimetry, radiotherapy, radiobiology and radioprotection. Measurement of the ionisation potential of non polar dielectric liquids.

Titre du projet n° 1 : Transport simulation of electrons, photons, and heavy particles by Monte-Carlo methods.

Chef du projet et collaborateurs scientifiques : J. P. PATAU, M. TERRISSOL, M. MALBERT, A. PIQUEMAL, M. LHERMINE, M. A. COMBES, C. BARNAUD, C. CAZES

Une simulation des créations de vacances en couches électroniques internes par les diverses interactions qui en sont la cause (effet photoélectrique, Compton, interaction électronique inélastique) a été établie. Les créations de photons de fluorescence et d'électrons Auger sont ainsi simulées, ainsi que le transport de ces particules.

Ainsi que nous l'avons montré, celui-ci ne peut être négligé lorsque la numéro atomique des atomes concernés est élevé.

Une simulation complète du transport des protons a été entreprise et presque terminée. Les interactions coulombiennes sont simulées au moyen des distributions de déflexion de MOLIERE et de KEIL, ZEITLER et ZINN, et des distributions de longueur de trajet de VAVILOV modifiée par SHULEK et de SYMON. Dans le domaine énergétique des échanges de charge, le pouvoir de ralentissement est déduit des travaux de BRICE.

Les interactions nucléaires élastiques sont traitées par simulation de la cascade intranucléaire : a) pour les noyaux de nombre de masse supérieur à 12 à l'aide du modèle nucléaire du gaz de Fermi, b) pour les noyaux de nombre de masse inférieur à 12, par l'étude de toutes les interactions élémentaires (cette étude est réalisée en fonction du temps).

La désexcitation du noyau résiduel est traitée à partir du modèle statistique du noyau et de la théorie de l'évaporation de WEISSKOPF. Ces interactions peuvent être étudiées pour des protons d'énergie inférieure à 50 GeV (au-delà nous ne disposons pas de toutes les sections efficaces).

La simulation d'une interaction fournit les caractéristiques énergétiques et directionnelles des particules (protons, neutrons,pions,particules α,...) sortant du noyau.

Le programme ainsi mis au point peut fournir toutes les caractéristiques physiques et géométriques d'un faisceau de protons, ainsi que les distributions des dépôts d'énergie et celle des événements ionisants.

Au cours des années 1976-80, nous avons, dans le domaine des basses énergies, mis au point un code de calcul Monte-Carlo qui permet d'obtenir les trajectoires très détaillées d'électrons de 30 keV se ralentissant dans l'eau à l'état liquide. En tout point des trajectoires simulées nous connaissons les spectres énergétiques et angulaires de toutes les particules primaires et secondaires mises en mouvement ou non et les distributions de tous les ions ou excitons créés. A partir de l'étude statistique de nombreuses trajectoires aléatoires, il est possible d'obtenir la plupart des résultats physiques intéressant la radiophysique, la dosimétrie, la radiobiologie, la microdosimétrie... Ce type de données est directement utilisable soit pour comparaison avec l'expérience, soit pour l'introduction dans des calculs théoriques. Nous avons plus particulièrement appliqué nos calculs à la radiobiologie pour le calcul de distributions de distances inter-

ionisations dans des volumes entourant l'ADN, ainsi qu'à la microdosimétrie pour des calculs de fonctions de proximité ou des distributions d'énergie spécifique ou linéale.

On peut voir par exemple sur la figure 1 les variations de la fonction de proximité différentielle (t) obtenues pour des électrons de diverses énergies initiales se ralentissant complètement dans l'eau à l'état liquide.

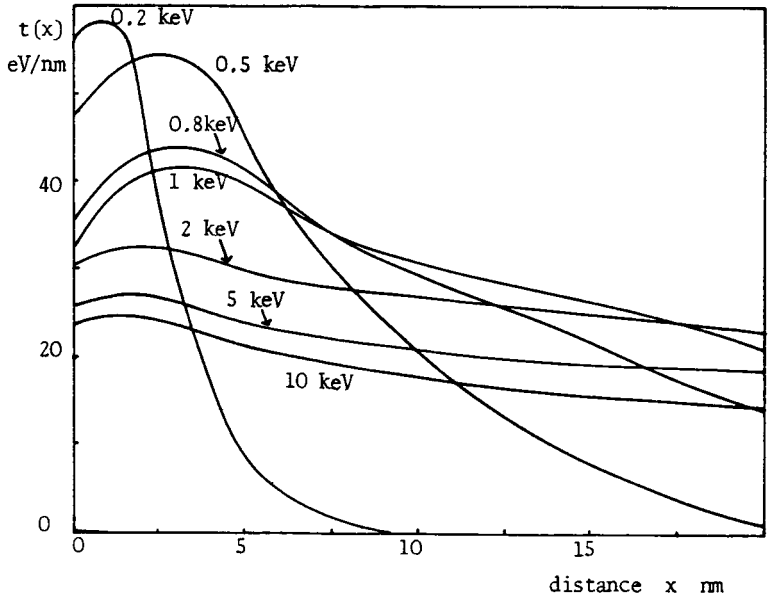

FIGURE 1 : Fonction de proximité différentielle t(x) pour des électrons dans l'eau liquide.

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- J. P. MANENS : Modèle de simulation du transport des électrons d'énergie inférieure à 30 keV. Application à quelques milieux gazeux. Doctorat de Spécialité, Toulouse, 1976.

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Titre du projet n° 2 : Measurement of the ionization potential of non polar dielectric liquids.

Chef du projet de collaborateurs scientifiques : J. CASANOVAS, D. DELACROIX, R. GROB, J. P. GUELFUCCI et R. LAOU SIO HOI.

A - RESULTS

All the work we have done in the framework of the European 1976-1980 Program on Dosimetry dealt with low energy photons ($7 \leqslant h\nu$ (eV) $\leqslant 10$) interactions in pure non polar liquids and in some solvent-electronegative compounds mixtures. Our objective was to get informations on the influence of the physical state of these compounds on their ionization potential values and on the interaction mechanisms between "electron scavengers" and solvent excited states or ion pairs(*).

1 - Liquid phase ionization potential values measurements

In 1980 we have measured the liquid phase ionization potential values, $I.P._l$, of 2,2-dimethylpropane (neopentane) and of tetramethylsilane. The results are given in table 1. These results, on two low dielectric constant liquids ($\varepsilon_r \simeq 2$), complete that already given in our previous reports and show that for these liquids the ionization potential values are lowered by about 1.6 eV in going from the gas phase to the liquid phase. It is obvious from these results that the effect of the condensation state of a target on its ionization potential value can't be neglected in an accurate simulation of low energy photons interactions in condensed media.

(*) This work is at present in process of extension to some polar liquids approximating to biological media.

TABLE 1 - Liquid phase ionization potential values.

Compound	$I.P._g$(eV) (**)	$I.P._l$(eV)	$\Delta I.P.$(eV)
2,2-dimethylpropane	10.35	8.55	1.80
tetramethylsilane	9.79	8.05	1.74

(**) $I.P._g$: gas phase ionization potential.

2 - Semi-empirical ionization threshold laws

More accurate $I.P._l$ values would be obtained if a theoretical law was known which would predict the photocurrent dependence on photon energy. As this is not the case for the present we have tried to fit our experimental results with some semi-empirical laws (exponential, power functions,...).

For all the liquids we have studied a good fit has been obtained with the following relationship :

$$\sigma \propto (h\nu - I.P._l)^{5/2} \qquad (1)$$

However for tetramethylsilane a better fit on a more extended photon energy range has been achieved with the following power function :

$$\sigma \propto (h\nu - I.P._l)^{3/2} \qquad (2)$$

This is shown in figure 1 for two compounds, 2,2-dimethylpropane and tetramethylsilane.

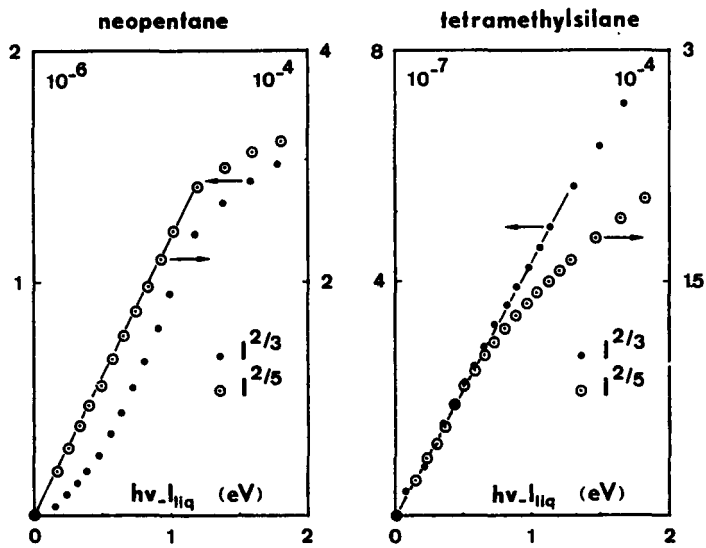

FIGURE 1 : Plots of $I^{2/3}$ and $I^{2/5}$ versus ($h\nu$ - $P.I._1$) for neopentane ($(P.I._1)_{2/3}$ = 8.55 eV, $(P.I._1)_{2/5}$ = 8.53 eV) and tetramethylsilane ($(P.I._1)_{2/3}$ = 8.05 eV, $(P.I._1)_{2/5}$ = 7.9 eV).

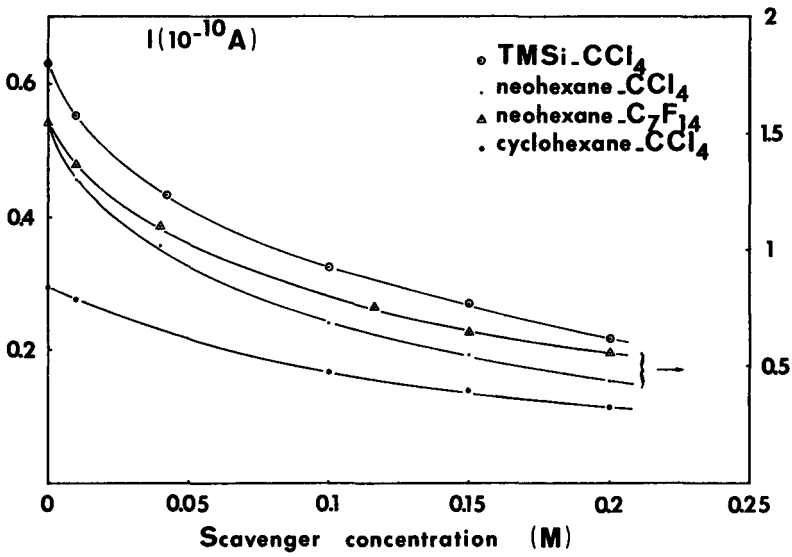

FIGURE 2 : Photocurrent dependence on electron scavenger concentration, c, for neohexane-CCl_4 ($\lambda_{ex.}$ = 129.6 nm), cyclohexane-CCl_4 ($\lambda_{ex.}$ = 130.5 nm), tetramethylsilane-CCl_4 ($\lambda_{ex.}$ = 147 nm) and neohexane-C_7F_{14} ($\lambda_{ex.}$ = 129.6 nm) mixtures. E = 15 kV.cm^{-1}, t° = 22°C, ϕ = 3.10^{10} quanta/sec.

3 - Effect of an electron scavenger on the photocurrent intensity

As anticipated in our work program, we have studied the effect of some electron scavengers on the solvent photocurrent intensity and we have compared these results to that already obtained on the same mixtures irradiated by high energy photons (^{60}Co γ rays).

Figure 2 shows the photocurrent (E = 15 kV cm^{-1}) dependence on electron scavenger concentration, c ($10^{-3} < c(M) < 2\ 10^{-1}$) for neohexane-carbontetrachloride (CCl_4), cyclohexane-CCl_4, tetramethylsilane-CCl_4 and neohexane-perfluoromethylcyclohexane (C_7F_{14}) mixtures irradiated, at 22°C, by respectively 129.6 nm, 130.5 nm, 147 nm and 129.6 nm wavelength photons.

We see that the introduction of an electron scavenger reduces drastically the solvent photocurrent, this effect is much important than that we have observed in the case of an irradiation by high energy photons.

The plots of I_o/I_c versus scavenger concentration give straight lines. The values of I_o (photocurrent in pure solvent) and of I_c (photocurrent at a scavenger concentration c) were deduced from figure 2. The values of the slopes of these curves are given in table 2. These values are independent from the excitation wavelength values (at least between 128 nm and 146 nm) as well as from the applied electric field strength (at least between 0 and 20 kV cm^{-1}).

The good linear regressions we have obtained for I_o/I_c versus c, indicates that at low excitation energies the scavengers react essentially with "extended excited states" of the solvents and not with the geminate ion pairs arising from these excited states decay, as it is the case at much higher excitation energies (^{60}Co γ rays irradiation for example).

TABLE 2 - Slope values deduced from the plots of the photocurrent quenching function I_o/I_c vs concentration of : a) CCl_4 in cyclohexane, neohexane and tetramethylsilane ; b) C_7F_{14} in neohexane.

Solute	Solvent	Slope from I_o/I_c vs c (M^{-1})
CCl_4	cyclohexane	7.5
	neohexane	13
	isooctane	9.5
	tetramethylsilane	9.5
C_7F_{14}	neohexane	9

B - CONCLUSION

The initial objectives of this project have been fully attained. It is obvious that a gap exists between the liquids we have studied so far and biological materials however, owing to the very few results existing on low energy photons interactions in condensed media and on liquid ionization potential measurements, our results present the advantage to show the importance (which would be even greater in biological materials) of the condensation state of a medium on its ionization potential value. This would entail a modification of the earlier steps picture of the ionization process. Our results show also that the interaction between some compounds and the irradiated medium depends on the energy of the radiation used and particularly that at low irradiation energies the molecular excited states play a prominent part.

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Vertragspartner der Kommission:	Gesellschaft für Strahlen- und Umweltforschung mbH München Institut für Strahlenschutz
Vertrag-Nr.:	211-76-1-BIO D
Leiter der Forschungsgruppe:	Dr. G. Burger, Prof. Dr. W. Jacobi
Allgemeines Thema des Vertrages:	Mikrodosimetrie und Neutronendosimetrie

The contractors research group "Radiation Physics and Neutron Dosimetry" in the GSF-Institute for Radiation Protection is predominantly engaged in radiation protection research.

The long term programme of the Laboratory for Radiation Physics and Microdosimetry in the group covers three main topics:

- experimental studies of electron and ion transport, especially of the ion track structure in matter and measurement of global dosimetric parameters, such as W-values.
- theoretical studies on the same subjects by development, improvement and application of radiation transport codes.
- the application of the physical information on the primary radiation effects on biological model targets.

It was the first two items which were presented in the programme proposal and were supported by the contract. In fact the third point could not be investigated to the proposed original extent. During the contract period our main interest changed from the short term development of microdosimetric models in the field of quantitative radiobiology to a better understanding of radiation risk to man especially at low doses. Any microdosimetric approach in this respect seems, however, extremely difficult. So we have concentrated on the improvement of our physical knowledge of radiation transport and charged particle interaction track structure.

Aside from these activities we were continuously engaged in neutron dosimetric aspects under the auspices of the CENDOS-committee, which was another item of the proposal.

The contract covered a continuous 12 man-month support per year, about 50 % of which was spent for the experimental track structure investigations and the rest shared roughly equally for the theoretical microdosimetric work and investigation of neutron beam dosimetry problems.

1. Experimental radiation physics

The following experiments were performed during the period 1967 - 1980 and were partially supported by the contract.

1.1 Measurement of dosimetric parameters

- Measurement of W-values of low energetic electrons: (/1/, /2/)

  The W-values were measured for monoenergetic electrons in the energy range from 5 to about 500 eV in air, N_2, O_2, CO_2, tissue-equivalent gases (Rossi-Failla and Srdoc mixtures), methane, ethane, propane, *n*-butane, i-butane, pentane, hexane, nonane, ethylene, acetylene, ethanol, acetone, H_2O, D_2O, H_2, D_2, C_6H_6, C_6D_6, argon, krypton, and xenon. The W-values of all gases increase continuously with decreasing electron energy; they approach infinity asymptotically at the ionization thresholds and the well-known energy-independent high-energy W-values at high electron energies. The experimental error is estimated to be less than 2 %.

- Measurement of W-values of low energetic ions: (/3/, /4/, /5/)

  The W-values were measured in the energy range from 500 eV to 50 keV for atomic and molecular hydrogen and nitrogen ions (H^+, H_2^+, N^+, N_2^+), and He^+, C^+, O^+, Ar^+-ions in nitrogen, air, carbondioxide, methane and methane based TE-gas. The results for atomic and molecular ions at the same energy per mass unit were practically identical. The results above 20 keV generally fit reasonably well to the data of Chemtob et al.(1978). In all cases, except one, they show monotonously falling curves with increasing energy. Only for H^+ in nitrogen the values at the highest energies measured (30 - 50 keV) are increasing slightly again, indicating the possible existence of a broad minimum between 10 and 50 keV. For protons the agreement with the few other data sets existing below 50 keV for some of the gases measured (Sidenius 1978, Boring 1965, Lowry 1958) is poor. For He^+ and the heavier ions the results agree very well with all existing data.

1.2 Track structure experiments

- Electron transport experiments:

An experiment was builtup which allows the measurement of electron spectra at arbitrary positions and with arbitrary orientation of the axis of a small multigrid retarding potential electron spectrometer probe within a large chamber. The chamber can be pumped or filled with gas and flanged to external electron guns as well as to accelerator ion beams. Electron transport problems were studied in earlier experiments by measuring electron degradation spectra

- behind carbon foils of various thicknesses irradiated with 1 keV electrons and
- in water vapour and nitrogen gas in the slowing down region of a 1 keV electron beam /6, 7/.

The differences observed between the spectra measured behind carbon foils and in water vapour for comparable geometries were found to be very small. The findings indicate that, apparently, phase state effects do not influence the electron transport properties of low Z materials to a large extent. In addition some experiments were performed in nitrogen gas to check to what extent the differences in electron collision cross sections influence the electron transport properties and how far calculations performed for water vapour can be scaled to other gases.

- Ion track experiments: (/8, 9/)

The same apparatus, as described above was used to measure the double differential electron distributions in the track of a 1 MeV proton beam passing through water vapour. The electrons were registered in an energy range from 1 eV to 2.6 keV in 450 different radial and angular positions. The radial distances range from 0.5 to 30 nm, when normalized to unit density. The data allow multiple interpolations, so that more global quantities can be calculated by integration over different parameters. The results provide considerable information about the electron energy transport mechanisms in the track of heavy ions. The flux of high-energy electrons is directed preferably away from the ion beam, while the flux of low-energy electrons is nearly isotropic. The intensity of the low-energy electron flux decreases faster with increasing radial distance than that of the high-energy flux, which indicates that the electron spectra are hardening with increasing distance.

In a second experiment, the spatial light emission density around ion beams in nitrogen gas was measured by means of an optical telescope /10, 11/. The light is emitted due to the excitation of molecules by energetic

electrons in the track. Two intensive nitrogen band series are the first negative and the second positive system. The (0.0) bands were selected by means of optical filters. The emission density varies by a factor of 10^8 in the distance range from formally 0.02 nm to 250 nm (after normalization of gas distances to unit density). It shows a similar course for the two bands, although their corresponding excitation functions are very different, the one showing a maximum at 17 eV electron energy the other at 80 eV. The shape and magnitude of the decrease of the spatial emission density with increasing distance depend on the proton energy.

2. Theoretical investigations on electron transport and ion track structure.

It has been speculated since Lea's early concept of "associated volumes", that it is the correlation between the spatial pattern of radiation events and the topology of the biological target, which describes radiation quality and determines the biological effect. Modern microdosimetry is hence investigating the detailed particle track structure by means of Monte-Carlo simulation calculations. It was the main aim of the project to develope such a Monte-Carlo-code and to compile and cross check all necessary input data (cross sections) for water as a model substance /12/. Transport calculations were then performed for electrons in the energy range from 30 eV to 3 MeV, and for protons in the energy range from .3 to 3.0 MeV The results were analysed primarily for the theoretical confirmation of the experimental results as described in chapter 1 /13/. In addition they were, however, applied to many other problems in radiation physics and quantitative radiobiology /14, 15, 16, 17/ and may finally lead to new descriptors of radiation quality beyond the concept of classical microdosimetry /18/.

3. Neutron beam dosimetry.

The increased installation of fast neutron therapy units as well as the growing interest in neutron radiobiological research necessitated intensive investigations in neutron and mixed radiation field dosimetry. This resulted in the following activities, which were again partly supported by the contract.

- Investigation of ion chambers

  In a first period the ion chambers used by our group were thoroughly investigated with respect to all interesting experimental correction factors /19, 20, 21/. In a second period this investigation was extended to other chamber types, in order to evaluate the applicability of a chamber system as a reference system within the European therapy and radiobiology centers /22/.

- Build up of neutron calibration facilities
  and performance of intercomparison studies
  The ENDIP-results were further evaluated, preliminarily published in 1976 /23, 24/ and 1977 /25, 26/ and finally published in 1978 /27/. As a consequence of diverging results of three European participants in the INDI and both ENDIP-sessions, a small scale intercomparison project was initiated under the sponsorship of the CENDOS-committee and performed at GSF /28/.

- Theoretical neutron transport studies
  The neutron and mixed radiation transport studies in homogeneous phantoms, by means of the transport code DOT, were continued mainly to study the spatial variation of radiation quality inside the phantom /29, 30, 31/.

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- Contractant de la Commission: Université Louis Pasteur-Faculté de Médecine - Laboratoire de Biophysique des Rayonnements et de Méthodologie - Formation de Recherche Associée N°26 de l'INSERM - 11, rue Humann 67085 Strasbourg Cedex (France).
- N° du contrat: 170-76-1 BIOF
- Chef du groupe de recherche: R.V. RECHENMANN.
- Thème général du contrat: THEORETICAL AND EXPERIMENTAL STUDY ON THE IONIZING ENERGY LOSS MODES OF CHARGED PARTICLES IN MATTER. CONSEQUENCES FOR MICRODOSIMETRY, RADIOBIOLOGY AND NUCLEAR MEDICINE.

- Titre du projet N°1: THEORETICAL AND EXPERIMENTAL STUDY ON THE IONIZING ENERGY LOSS MODES OF CHARGED PARTICLES IN MATTER. CONSEQUENCES FOR MICRODOSIMETRY, RADIOBIOLOGY AND NUCLEAR MEDICINE.
- Chef du projet et collaborateurs scientifiques: R.V. RECHENMANN, E. WITTENDORP-RECHENMANN, B. SENGER.

A. INTRODUCTION.

In the contract period considered (1976-1980), the results of track analytical studies of the Laboratory, which had been reported previously, e.g. the presence of relatively energetic secondary electrons, even in the lower energy region of an α particle crossing complex media, as well as the relatively high occurrence of energetic recoil nuclei/ions, have been confirmed experimentally. In the same line of thoughts, various theoretical approaches, classical or quantum mechanical, have been undertaken in order to interprete experimental cross-sections determined in the Laboratory or derived from the literature. As a logical consequence of these investigations, a study on the range-energy relation of medium energy heavy charged particles in tissue-like media has been undertaken in connection with the evaluation of the contribution of the electronic and nuclear stopping powers to the energy loss of these particles in biological tissues.

B. LIGHT SECONDARIES.

Linear density distributions of ejected secondary electrons have been determined on high populations (statistics: $\sim$ 1000 tracks) of α particle trajectories with energies comprised between 4 and 12 MeV recorded in nuclear track emulsions at various concentrations of the CNOH compound.

An interpretation of these experimental data has been attempted by means of various differential cross-section formulas proposed by the literature (1,2). An acceptable agreement along the whole energy range of the incoming particle could not be obtained, whatever the ejection angle θ or the detection threshold $\bar{T}_0$ chosen; furthermore, the slope of the corresponding production curves was systematically too steep if compared with the experimental yields (Fig.1a,b, 3a,b).

A better agreement with the experimental data could be obtained by the so-called modified mixed treatment (MT), where a quantum mechanical expression (PWBA) and a classical one (Rutherford, BEA) were combined in order to treat all the shells and subshells of the atoms constituting the medium.

The importance of the threshold energy of the secondaries as a parameter, which appeared in these studies, as well as the necessity of a description of the spatial distribution of the ejected electrons around the incoming heavy charged particle, led us to introduce expressions of double differential cross-sections (DDCS) in our calculations. Here again, the direct application of classical (3) or quantum mechanical (4,5) DDCS from the literature did not allow to reproduce our experimental data over the whole energy range considered (Fig.2,4). We have therefore attempted to introduce in the expression of the DDCS, developed for the particular case of protons bombarding hydrogen, expressions of Merzbacher (6,7,8) describing the screening effect for the K, L and M shells and subshells. This approach led to the development of two related expressions, one being a specific development starting from a basic formula of Landau (9).This formalism can be completed by the introduction of the classical DDCS for the N shell and following shells. By means of this DDCS-MT, a good agreement could be obtained with the δ-ray production measured in the three ionographic detectors L4x1, x2, x4 (Fig.5a,b,c) for corresponding values of the track width, which could effectively be verified experimentally in the three detectors considered.

The comparison between experimental angular distributions (Fig.6)(10) as well as ionization cross-sections integrated over all angles and energies (Fig.7)(11), and the values calculated by means of the DDCS-MT could be in most cases considered as satisfying, and that without the introduction of any fitting parameter.

This DDCS-MT is relatively easy to program. Nevertheless a study has been undertaken in order to establish an even more simplified set of formulas, which is intended to be a reliable and flexible tool for the determination of the energy distribution around the tracks of heavy charged particles crossing tissue-like media.

C. HEAVY SECONDARIES.

In the frame of our investigations on the ionizing pattern surrounding the trajectory of heavy charged particles traversing tissues, the systematic study of thick protuberances sticking out of the primary track core has been continued. These secondary branches were at least partly interpreted in terms of short tracks of nuclei of the atoms constituting the tissue-like medium ejected by the incoming particle (Fig.15a,b).

The morphological analysis as well as the counting of this type of secondary events has been performed by combining direct visual observations with a semi-automatic image analyzing system repeatedly on high populations (statistics on 1000-8000 primary tracks) of medium energy α particle tracks ($E_\alpha \leq 12$ MeV) recorded in L4 nuclear track emulsion at various gelatin concentrations. Severe selection criteria have been introduced in these determinations in order to exclude any possibility of a contamination by spurious photographic effects.

The working hypothesis that a large part of the observed thick protuberances are short tracks of recoil nuclei has been confirmed by the measurement of the difference $\overline{\Delta R}$ between the mean track length $\overline{R}_b$ of recorded 12 MeV α particle trajectories without any

visible protuberances, and the mean range of tracks $\overline{R}_r$ with visible detectable thick branches, which allowed to evaluate the mean energy difference $\overline{\Delta E}_{exp.}$ between the two populations, which was effectively positive (Table I). A mean energy loss $\overline{\Delta E}_{calc.}$ of the same order of magnitude could be estimated on the basis of theoretical considerations related to the Sigmund formalism (Section D). The introduction of the stopping powers of LSS and Andersen-Ziegler in this formalism resulted, for a radial threshold $\hat{r}$, in the values given in Table I under (a) and (b), respectively.

TABLE I.

Emulsion	σ_{R_b} (μm)	$\hat{r}$ (μm)	σ_{R_r} (μm)	$\overline{\Delta R}$ (μm)	$\overline{\Delta E}_{exp.}$ (keV)	$\overline{\Delta E}_{calc.}$ (keV)	
						(a)	(b)
L4x1	1.55	0.43	3.61	2.87	∿ 440	∿ 500	∿ 300

It can also be seen in Table I that the standard deviation σ_{R_b} and σ_{R_r} corresponding to the smooth tracks and to the trajectories with protuberances, respectively, are significantly different. A strong increase in the dispersion of the ranges has effectively to be expected in the case of energetic collisions of the incident particle with nuclei of the medium.

Furthermore, if $\overline{\Delta R}$ and the corresponding $\overline{\Delta E}$ is represented as a function of the length L of the recoil tracks, it appears that the trend of $\overline{\Delta R}$ ($\overline{\Delta E}$) is to increase with L (Fig.16); the averaging of $\overline{\Delta R}$ ($\overline{\Delta E}$) for all the tracks with protuberances above a given value of L shows the same trend (Fig.17).

It could also be shown that the production of very heavy secondaries (C,N,O) for a given radial distance from the track axis in two detectors with two different concentrations of the CNOH compound is proportional to the gelatin concentration.

The calculation of the production of heavy secondaries has been attempted by means of a formalism proposed by Sigmund (12), which takes into account the electronic stopping power for which an expression given by Ziegler was used. This production has been compared with experimental data by making use of the corresponding ranges determined by the approaches of Lindhard, and Andersen and Ziegler, respectively (see Section D). Depending on the range-energy relation chosen and on considerations on the emulsion geometry, the calculated number of recoil nuclei ejected by 8 MeV α particles crossing L4x1 emulsion can be estimated between 1% and 3% (Fig.19). This value is of the same order of magnitude as the production of ∿ 7% determined experimentally. The same procedure has been applied in order to calculate the production of protons ejected by 8 MeV α particles traversing L4x1 emulsion (Fig. 18).

The extrapolation of these results to the lower energies T, i.e. having a radial spread $r > 50$ Å, would indicate the ejection of ∿ 30 protons and ∿ 3 C, N and O ions, hence ∿ 33 recoil nuclei by a 12 MeV α particle crossing gelatin. Considering the fact that these heavy secondaries will themselves eject nuclei out of a tissue-like medium (formation of arborescences and cascades), the possibility of a contribution of this mode of energy loss to radiobiological effects may be taken into consideration.

D. PRELIMINARY RANGE-ENERGY DETERMINATIONS OF HEAVY CHARGED PARTICLES.

A general study on the stopping power and the range of intermediate and low energy heavy ions in organic media has been started. Nuclear track emulsions which can be considered simultaneously as a detector and as a target have been used for these investigations.

The influence of the so-called activation development procedure on the first and second order moments of range of α particles recorded in Ilford K2 and L4 emulsions has been compared with the data obtained by means of a classical development (Fig.8). The activation procedure resulted effectively in a significant increase in range as well as in a decrease of the standard deviation of the range distribution. It was therefore interesting to reconsider the measurement of these track parameters in ionographic detectors submitted to a latent image activation process.

A tentative evaluation of the contribution of the nuclear stopping power to the total energy loss has been undertaken by means of differential range-energy determinations on populations of α-tracks with and without visible events (see Section C and Table I).

Theoretical determinations of ranges have been attempted in the medium and in the low energy region of an incident heavy charged particle crossing ionographic detectors. The first order moments of range of Helium nuclei have been calculated by means of a semi-empirical determination given by Barkas (13), and by an approach based on a scaling procedure given by Ziegler (14) applied to the proton electronic stopping of Andersen and Ziegler (14).

The ranges of the C, N, O, Br and Ag ions with energies ranging from 1 - 1000 keV, and for H-ions with energies ranging from 1 - 100 keV have been estimated on the basis of the stopping power data and scaling factor given by Andersen and Ziegler (14), as well as by means of the formulas developed in the frame of the LSS theory (Fig. 9-14).

E. CONCLUSION.

These results taken as a whole allow already to perceive the preliminary shape of what could be called the ionizing track printed in a tissue-like medium. The research actually under way in the Laboratory is intended to go into more detail of the ionizing track pattern, e.g. of the form of the interaction cylinder generated by the low and medium energy secondary electrons (δ-rays, Auger's electrons), as well as to evaluate the contribution of the first, second and n^{th} recoil nuclei generations to the destructive effects of heavy charged particles crossing biological tissues.

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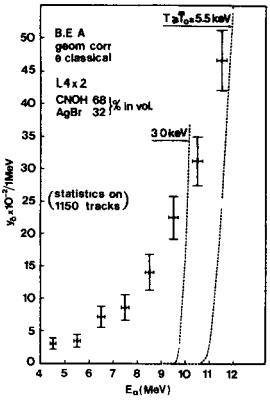
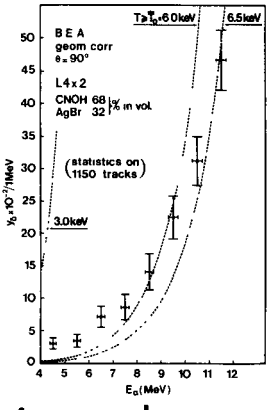


a

Fig.1



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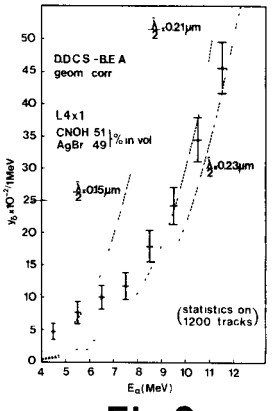

Fig.2

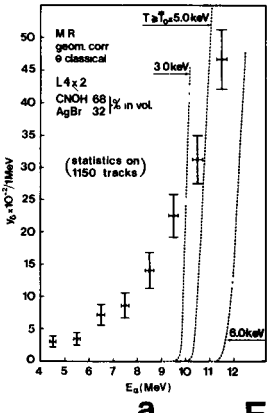

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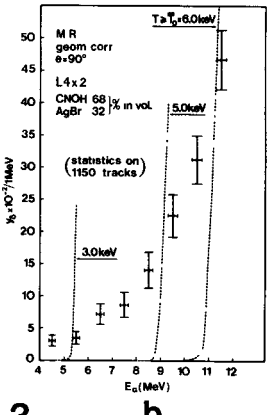
Fig.3



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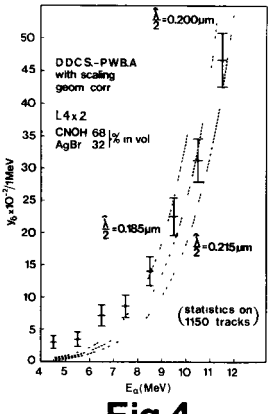

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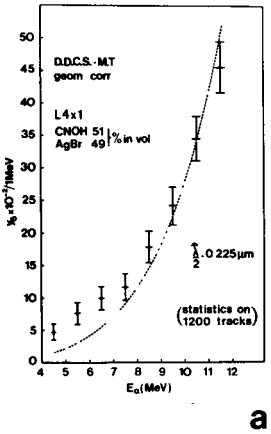

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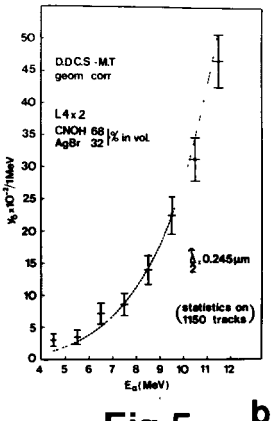
Fig.5



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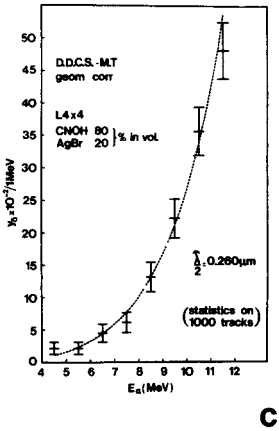

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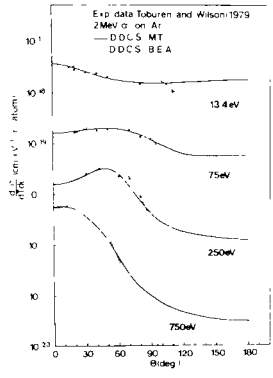
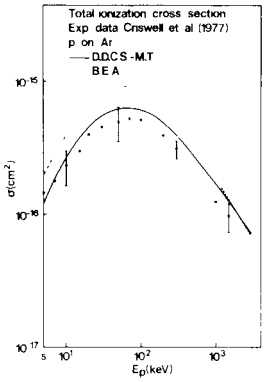


Fig.6



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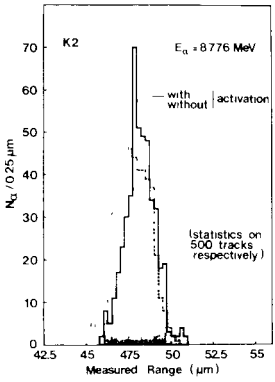


Fig.8

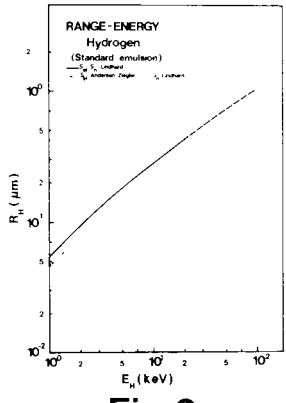


Fig.9

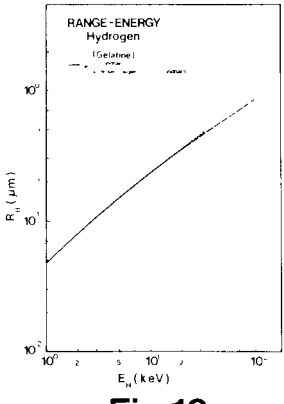


Fig.10

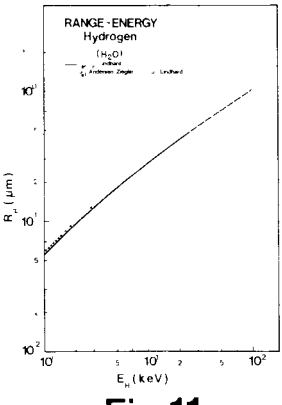


Fig.11

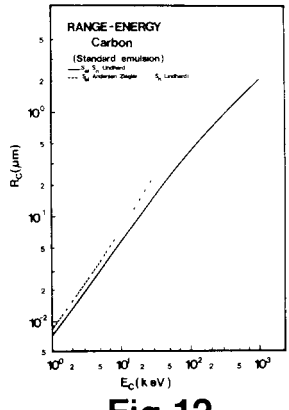


Fig.12

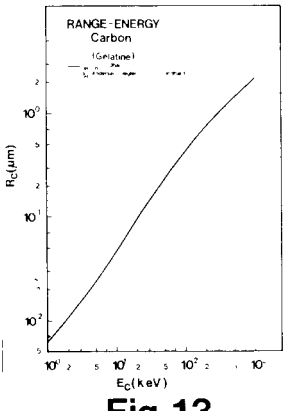
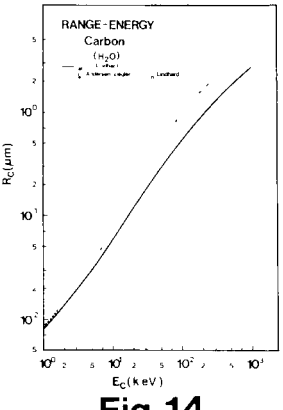


Fig.13



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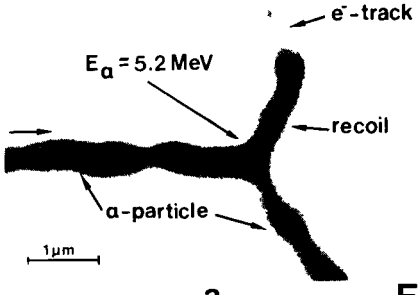

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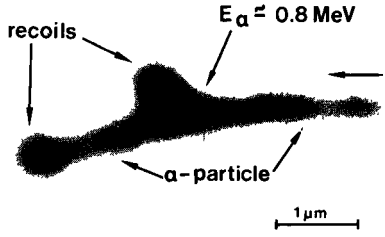

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Fig.15

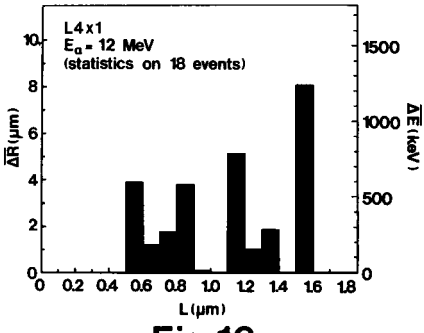

Fig.16

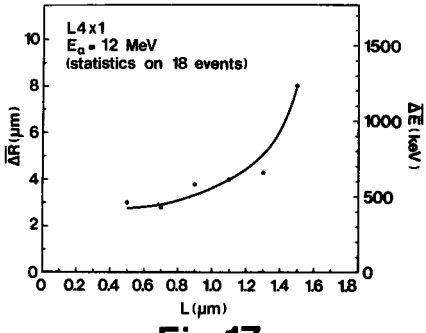

Fig.17

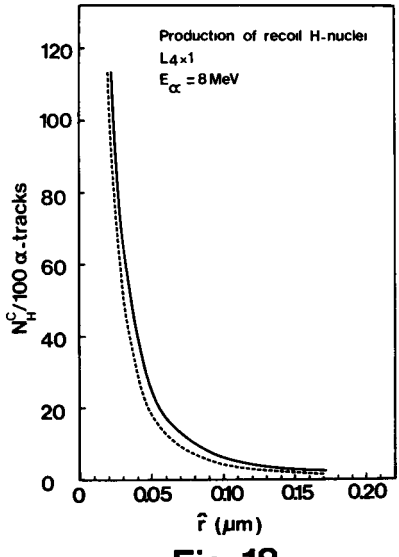

Fig.18

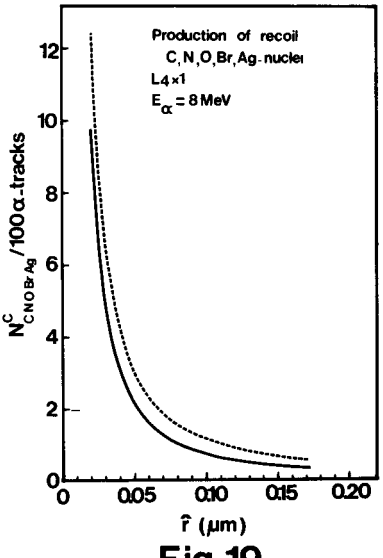

Fig.19

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Vertragspartner der Kommission: Universität des Saarlandes, Saarbrücken BRD

Nr. des Vertrags: 21o-76-1 BIO D

Leiter der Forschungsgruppen: Prof. Dr. H. MUTH, Prof. Dr. R. GRILLMAIER

Allgemeines Thema des Vertrags: Microdosimetric and EPR-investigations of energy transfer and induction mechanisms of radiation damage using model substances and human lymphocytes together with studies of special types of radiation induced biological damage in lymphocytes.

Titel des Projekts Nr. 1: EPR-measurements of solutions (irradiated by X-rays and neutrons) with increasing numbers of biologicaly interesting components and scanning electron microscopic studies of metaphase chromosomes in irradiated human lymphocytes.

Leiter des Projekts und wissenschaftliche Mitarbeiter:
Prof. Dr. R. GRILLMAIER, L. BIHY (1976-1979), Dr. FELL (1976),
W. SCHMIDT (1977-198o), H.-K. STANGER (1978-198o)

The most effective radiation protection is provided by a profound knowledge of the induction mechanisms of biological radiation damage. The induction mechanisms can be divided into four sections: energy deposition, physico-chemical and chemical processes and finally biological effects. Generally in the past processes of each of the four sections have been investigated separately, whereas we try to study representative reactions of the four phases under identical or at least comparable conditions, in order to get comparable results and to obtain insight into the correlation of the different processes of the induction mechanism.

Project No. 1 comprises investigations of the physico-chemical and chemical phase as well as of the biological (especially at low temperature), wheres project No. 2 deals particularly with the processes of energy deposition. Further investigations at the biological level are performed in close cooperation of the two groups.

Ia. Radicals are considered to be the most essential radiolytic products created in the physico-chemical and chemical phase of radiation action. Therefore studies of these intermediate products in cells and especially in model substances had been planned.

Previous investigations showed the absence of free electrons and hydrogen radicals in samples (containing water) irradiated and measured at about 8o K, giving evidence that even at this low temperature the free electrons as well as the H-radicals undergo reactions. Therefore the problem had to be investigated whether or not these radicals cause an indirect radiation damage even at this low temperature. The investigations have been performed using

a radical scavenger having great affinity to e^- and H'. The results of EPR measurements indicated that there is no qualitative nor quantitative difference between the radicals induced in water samples with and without radical scavengers. Therefore must be concluded that reactions between the electrons and the H'-radicals reduce their concentration preventing this portion of the indirect radiation effect.

Ib. Chromosome aberrations seem to be an informative reaction of the biological phase near the molecular level of radiation action.

Examinations of human lymphocytes irradiated also at 77 K by X-rays resulted in chromosome aberration rates (total number of single breaks) reduced by a factor of 15 compared with the aberration rate after irradiation at room temperature. (The reduction factor for dicentrics is 45.) Considering only the results of radical investigations described above, the indirect effect due to free electrons and H-radicals (induced in watermolecules) should be more than 90% of the radiation damage. At low temperature however the recombination of broken chromosome strands is probably favored by their immobility, contributing this way at a certain degree to the reduction of the aberration rate. Therefore further studies are necessary to determine the portion of the indirect effect more precisely.

II. The examination of human lymphocytes irradiated at 77 K by X-rays had been completed by investigations of cells irradiated at 4 K and 277 K by X-rays as well as tritium betas. Using these data the dose relation ship has been fitted by the linear-square function $y = \alpha D + \beta D^2$ according to the dual-radiation-action theory (D: dose). The parameters α and β and the results of microdosimetric measurements have been applied to calculate the "site diameters" d.

The site diameters evaluated for the total number of single chromosome breaks and for two break aberrations observed in cells after irradiation at 4 K and 77 K are about equal in magnitude. The site diameters observed for single breaks and dicentrics after irradiation at 4^oC are about two to three times the diameters obtained after irradiation at 77^oK for X-rays and tritium betas. Using the site diameters the "sentitive volumes" (spheres) have been calculated. Due to the reduction factors of 15 and 45 the ratios of the sphere volumes (at 4^oC to that at 77 K) are about 2o and 4o for single breaks and dicentrics plus rings.

Using the EPR-technique, the number of radicals have been measured in frozen DNA-solutions of appropriate concentrations after irradiating the samples with X-rays. The dose relationship for radicals has been evaluated. Applying

this relationship the numbers of radicals in the "sensitive volume" have been calculated per unit of absorbed dose assuming that for low LET radiation the radiolytic products are distributed uniformly throughout the irradiated sample. At doses giving rise to a mean aberration rate (single breaks) of about one per cell for example,surprisingly large numbers of radicals are produced. Obviously these radicals not only attack the chromosomes but also other cell compounds and cell organelles producing latent damages not seen by chromosome scoring. Because the cell membrane is one of the most important part of a cell, regulating especially the in- and outflow, the permeability of human lymphocyte membranes has been investigated using the trypan blue dying technique. (known as "viability test"; the dye only penetrates the membranes if the cell is no more "alive".) The in-vitro investigations have been performed applying radiations of low and high LET at various dose levels. A reduced survival rate has only been detected (by dyeing technique) for doses of more than some thousand rads beyond the scope of practical radiation protection. No measureable effects have been observed up to dose levels producing high aberration rates. The reduced rate of mitosis at this level, as reported some times, therefor cannot be explained by severe membrane damages.

III. In order to find out the type and amount of the radicals contributing to radiation damage (especially chromosome aberrations) two complementing methods of investigation have been developed and applied.

1. Investigations of radicals induced in pure water and water with an increasing number of solved nucleus compounds up to chromatin have been initiated, using the EPR-technique to determine the contribution of these compounds to the EPR-spectra. As the samples are irradiated and measured at low temperatures it is possible to observe the behaviour of the radicals at increasing temperature. The investigations are performed applying low and high LET radiation to establish potential differences of the radical behaviour. Investigations of water and solutions of DNA and a few other compounds have been already carried out. The procedure of extracting chromatin from calf thymus cells has been introduced and purified chromatin is now available in sufficient quantities.

2. Hard- and software for computerized managing of EPR-measurements and spectrum analysis have been developed.

The hardware enables the transfer of data and logical instructions between the EPR-spectrometer and the digital computer (PDP 11/4o).

The software consists of different packages enabling the user to separate complex additive spectra into the single lines (produced by a mixture of different radicals for example), and supply all parameters identifying the signals such as line shape, line width and especially g-values. Furthermore the programmes are capable to integrate the spectra as a whole as well as the single lines to obtain an index for the radical concentration. Applying the new programmes to analyze spectra of water samples irradiated at 77 K by X-rays, it could be demonstrated that the dependance on time of one of the three lines differs considerably. It is concluded, that there are at least two different kinds of radicals present in ice rather than only one as has been assumed to date.

IV. From the findings of chromosome aberration investigations we try to extract parameters, which are comparable with the results of EPR measurements. Such a parameter could be p, the fraction of cells getting an aberration per unit of time and dose. (p could also be considered as the probability of getting an aberration)
If n_k and n_{k-1} are the numbers of cells having k and k-1 aberrations, respectively, then the variation of n_k per unit of time is

$$\frac{dn_k}{dt} = p_{k-1} \cdot n_{k-1} - p_k \cdot n_k \qquad (1)$$

(p_i is the probability of producing an aberration in a cell having allready i aberrations, t is the irradiation period. Equation (1) includes the condition, that no more than one aberration is produced at the same time in a cell which at least is true for low LET irradiation and at low dose levels.)
Assuming, that p_i is independent of the number of aberrations existing already in the cell the Poisson function $n_k = \frac{n}{k!} (p \cdot t)^k \cdot e^{-pt}$ is the solution of eq (1). (n is the total number of cells irradiated.)
If the p_i are different in magnitude another solution is derived:

$$n_k(t) = n_o(o) \cdot \prod_{l=o}^{k-1} p_l \cdot \sum_{j=o}^{k} \frac{e^{-p_j t}}{\prod_{\substack{k=o \\ k \neq j}} (p_k - p_j)}$$

In 189 cases the distributions of aberrations among the cells have been compared with the Poisson function. For dicentrics the measured distributions may be described by the Poisson statistic in practically all cases and independent of the type of radiation used (X-ray, ^{60}Co-gamma, rays, tritium-betas and neutrons).

For fragments significant deviations from the Poisson distribution have been observed except after irradiation at 4 and 77 K by X-rays.

The equations (2) have been used to calculate the parameters p_i for fragments not in accordance with the Poisson statistics observed at low and moderate doses. The mean value of all observed p_o is significantly less than the mean of p_1 wheraes the means of p_1 and p_2 are equal in magnitude. The true reason of this phenomenon is not known but considering the large number of radicals necessary to produce a chromosome break, it may be due to damage of the repair system, occuring simultaneously with the first chromosome break in a cell.

V. Investigations of blood samples of radiotherapy patients treated by ^{60}Co-gamma rays have been started (but not yet finished) in order to study the relationship of radiation dose and chromosome aberration rate also in vivo. Additional chromosome investigations for radiologically examined children have been performed. The radiation field size and the actual surface radiation doses of each patient have been measured and used to calculate the averaged "whole body" exposure dose. The mean applied dose is 2.o rad. The mean aberration rates are o.o3 and o.oo3 per cell for acentrics and dicentrics respectively. These studies will be completed by in-vitro investigations in the low dose range. Blood samples of three human donors have been irradiated and cultured up to now, applying X-ray doses of 1, 2, 4, 8 and 1o rad.

VI. In cooperation with the working group of project No. 2 investigations of the chromosome aberration rates in mixed neutron-gamma fields of different radiation qualities have been carried out. Comparative studies of the aberration rates induced in human lymphocytes by neutron fields of different irradiation facilities have also been performed. These studies provide results which at least are useful for the practical purposes of radiation protection. (The results are described in project No. 2 in more details)

VII. Investigations of metaphase chromosomes using the scanning electron microscope technique have been stopped after first initial examinations. The information provided by SEM pictures seem to be at the same level as obtained by light microscopy. Furthermore it was not possible to examine the large number of slides and metaphase preparations because we do not have a SE microscope at our own disposal. Instead of SEM studies the exploration of chromosomes by light microscopy (reported above) have been carried out.

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GRILLMAIER, R.E., E. MAYER, "Chromosome aberration rates in glyerol preserved human lymphocytes after X-irradiation at 77 K, 4 K and at roomtemperature"

GRILLMAIER, R.E., "Relative biological effectiveness of tritium beta rays after irradiation at 77 K and +4°C estimated by the chromosome aberration rates.

GRILLMAIER, R.E., "Site diameters of chromosomal lesions due to dual radiation action theory and DNA radicals both induced by X-rays and tritium beta"

GRILLMAIER, R.E., C. BILIOTET, H. FELL, "Radicals induced in biological model substances at low temperatures by X-rays and alphas"

Titel des Projekts Nr. 2: Physical and biological aspects of the biological effectiveness of neutron and mixed radiation fields

Leiter des Projekts und wissenschaftliche Mitarbeiter:
Dipl.-Phys. H. G. MENZEL, Dipl.-Phys. L. BIHY, Dipl.-Phys. H. SCHUHMACHER, Dr. A. J. WAKER

Microdosimetric investigations have been performed in beams of radiations having a wide range of radiation qualities including neutrons of different energies, mixed neutron gamma fields, photons, fast electrons and negative pi-mesons. One aim of these studies was to examine in correlated radiobiological experiments the suitability of microdosimetric parameters for the specification of radiation quality in practical situations such as radiation protection. Additional microdosimetric measurements have been performed to investigate specific aspects of radiation protection and mixed field dosimetry.

Radiobiological experiments have been carried out in mixed neutron-gamma fields and with ^{60}Co gamma-rays jointly with the working group of Project No. 1 and research groups from other Institutes. The biological endpoints studied were chromosome aberrations in human lymphocytes and reduction of the colony forming ability in several mammalian cells after irradiation in vitro at 37^{o}C. Radiation fields of different neutron energies and varying contributions of gamma-rays to absorbed dose were achieved by irradiating cells in different positions with regard to the collimated fast neutron beams within water phantoms. The two sources employed were cyclotron produced neutrons having a broad energy spectrum ($\bar{E}_n$ = 8.5 MeV) and a 14 MeV D-T generator. The radiobiological results showed that at a given fast neutron source the biological effectiveness does not vary substantially within the whole water phantom not even outside the primary beam. This can be understood qualitatively on the basis of the measured lineal energy spectra. These

revealed that there are no marked changes with depth in phantom and that a decrease of neutron energy due to neutron scattering is accompanied by an increase of the photon dose fraction outside the direct beam. These changes have opposite and therefore compensating effects on the biological effectiveness. The radiobiological results obtained at two neutron sources differed from each other according to the different primary neutron energy.

The analysis of microdosimetric parameters and their dependence on irradiation positions revealed that y^*, the dose mean lineal energy corrected for saturation as suggested by KELLERER and ROSSI, has a closer correlation to the relative changes of the biological effectiveness found in the biological experiments than other parameters. There are indications that the agreement in terms of relative changes is improved if neutron and photon dose fractions are given separately and y^* is evaluated for the neutron component only. This result is of interest for radiation protection because it supports suggestions with regard to the suitability of microdosimetric parameters for applications in radiation protection. Moreover, these results suggest that in case of mixed radiation fields y^* may be a useful radiation quality specification for practical purposes. Obviously that does not imply that this or any other microdosimetric parameter is sufficient to predict the biological effect for any type of radiation and any endpoint. Also it has to be considered that within the project parameters influencing radiobiological effects could only be varied within certain limits and that the precision achievable in radiobiological experiments is limited, in particular at low doses. Because of these restrictions and because of the basic importance of these results such correlated experiments should be continued and extended to other biological endpoints.

The microdosimetric data obtained within the project are being used also by other research groups for the analysis and interpretation of their radiobiological results. (for instance by Abteilung für Biophysikalische Strahlenforschung, Gesellschaft für Strahlen- und Umweltforschung (GSF), Frankfurt (Prof. Pohlit)).

Specific problems in mixed field dosimetry have been investigated employing the technique of experimental microdosimetry and taking advantage of the detailed information contained in microdosimetric spectra. For example, it could be determined that the uncertainty in the total absorbed dose determination using tissue-equivalent proportional counters is comparable to

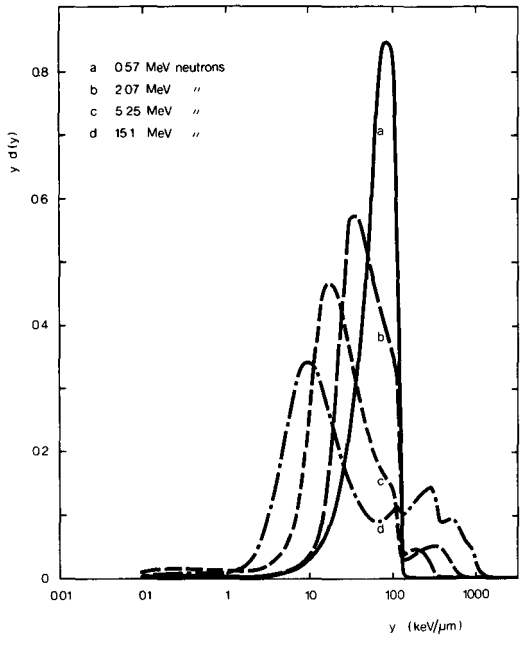

Fig. 1: Dose distribution per logarithmic increment of lineal energy for monoenergetic neutrons (simulated diameter d_s = 2 µm). The spectra have been measured at Gesellschaft für Strahlen- und Umweltforschung (GSF) in München

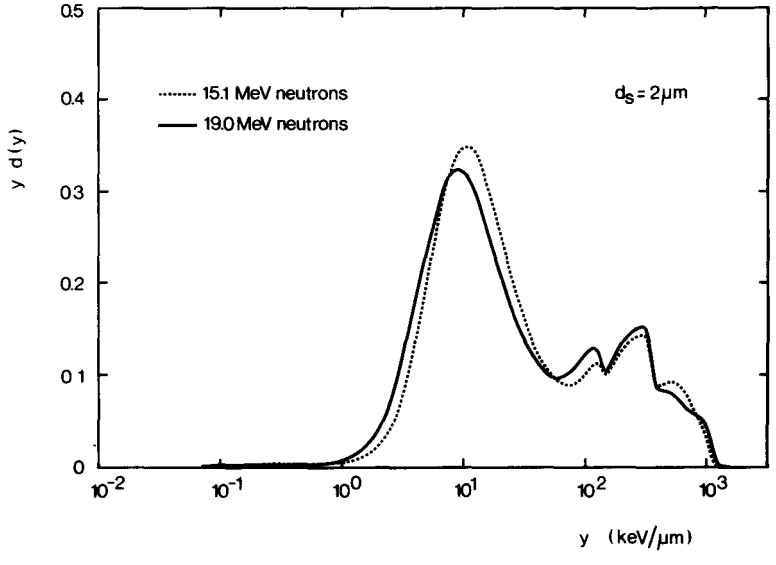

Fig. 2: Dose distribution per logarithmic increment of lineal energy for 15.1 and 19.o MeV monoenergetic neutrons (d_s = 2 µm). The spectrum for 19 MeV has been measured at Physikalisch Technische Bundesanstalt (PTB) in Braunschweig.

that of ionization chambers. Therefore this instrument has been used as a suitable dosimeter at very low dose rates. Microdosimetric spectra have been used also to separate neutron and photon fractions of total absorbed dose. It could be demonstrated that this method is associated with comparably small uncertainties even in case of lack of knowledge of the neutron energy. As the method is quite laborious it can only be recommended as a reference method for calibration of routine dosimeters and for the determination of the neutron sensitivity of nonhydrogeneous detectors. These results are important contributions to radiation protection dosimetry and practical beam dosimetry for radiobiological experiments in mixed fields as described above.

Practical aspects of the determination of effective quality factors and dose equivalent have been studied. For complex radiation fields various possibilities have been investigated to determine the effective quality factors directly from lineal energy spectra. It could be shown that functions can be given which can be applied directly to the measured lineal energy spectra and give effective quality factors for mixed radiation in good agreement with more conventional methods. These investigations are being continued and will be extended to monoenergetic neutrons up to 2o MeV.

In order to study systematically the uncertainties involved in experimental microdosimetry measurements were carried out in standard radiation fields such as monoenergetic neutrons and monoenergetic photons. In Figures 1 and 2 results obtained for monoenergetic neutrons between o.57 and 19 MeV are shown. These investigations have shown that such spectra also can be used for critical tests for calculations and their input data relevant to neutron dosimetry. These practical aspects of experimental microdosimetry will be studied further within the new research project.

The original aims of the project have been largely achieved. The results obtained indicate that quality functions dependent on microdosimetric quantities are suitable radiation quality specifications for radiation protection purposes. This is of practical interest because quality factors defined as function of microdosimetric quantities would allow to measure effective quality factors and dose equivalent directly using the well established techniques of experimental microdosimetry. A further important contribution to problems in radiation protection is that the applicability of tissue-equivalent proportional counters to specific aspects of neutron and mixed field dosimetry could be shown.

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Contractor :	National Radiological Protection Board, Harwell, Didcot, Oxon OX11 ORQ, England.
Contract nr :	164-76-1 BIO UK
Head of research team :	Dr. J.A. Dennis
General subject of contract :	Underlying parameters in dosimetry.

Title of project nr 1 :	Extension of the neutron cross-section data on hydrogen, carbon, nitrogen and oxygen upto 50 MeV energies.
Scientific collaborators :	Dr. J.A. Reissland, Mr. P.J. Dimbylow.

The paucity of experimental neutron cross-section data between 20 and 50 MeV for elements of biomedical interest (except for H) has prompted the use of nuclear model calculations to provide the required cross-sections. The objectives of the project were:-

(i) An assessment of existing nuclear models to identify those relevant to the energy and mass region.

(ii) The application of suitable nuclear models in conjunction with the scarce experimental data to produce cross-sections for the main elements of tissue, ie. H, C, N and O and also for other elements of biomedical importance, namely Mg, Aℓ, P, S, Ar and Ca.

(iii) The conversion of particle and recoil nucleus energy spectra for the above elements into kerma values.

The optical model represents the neutron-nucleus interaction by a complex optical potential. The real part of this potential represents scattering and the imaginary part describes nonelastic processes. A computer program, NOPTIC (Nucleon OPTICal model) has been developed. NOPTIC has been used to fit the experimental total cross-section between 20 and 50 MeV and ENDF/B-IV elastic and nonelastic data below 20 MeV. The calculated nonelastic cross-sections obtained were then used to normalise individual reaction cross-sections calculated by the statistical model.

The main reaction mechanism in this energy range is the statistical decay of the compound nucleus. However, the emission of the first particle from a target nucleus before statistical equilibrium is attained (precompound emission) becomes increasingly prominent as the neutron energy rises. Direct reactions are also important in the excitation of low lying levels, particularly for the (n,n′), (n,p) and (n,d) reactions. The first approach to the problem was to ignore precompound emission and direct reactions, also

low lying energy levels were not treated discretely in the statistical model but as part of a continuum of levels. The program SMOLDERS (Statistical Model Of Level Densities for Evaluating Reaction cross-Sections) was developed to calculate the emission probabilities of n, p, d, t, h and α particles for reaction chains of up to 6 sequentially produced compound nuclei. It is assumed that γ-emission is negligible above the threshold for particle production. The Fermi gas level density function is used and this is derived as the total state density for a system composed of two types of particle with equidistant non-degenerate single particle levels. The method applied in calculating reaction cross-section for C, N and O was to fit known reaction data by adjustment of level density parameters and then use these values to predict cross-sections for reactions which have no experimental data. A detailed description of this fitting process and the results obtained is given in Dimbylow (1978a).

A version SMOLDERS-K of the program SMOLDERS has been developed to convert centre-of-mass exit channel energies produced by the evaporation model into particle and recoil nucleus spectra, in the laboratory system, from which kerma values can be obtained. Multiple particle cascade chains (eg. ^{16}O (n, np np)^{13}C) are treated as sequential two-body break-up reactions. In all processes the angular distribution of the emitted particles is assumed to be isotropic in the centre-of-mass system. The emission of the first particle is treated using the standard kinetics for a binary reaction. The sequential emission of further particles is treated as though the compound nucleus collides with a massless target. The reaction kinetics in this case are complicated by the compound nucleus having a distribution of kinetic energies. Thus the laboratory spectra must be obtained by summing the individual laboratory distributions produced from all possible couplings of the compound nucleus kinetic energy with the reaction channel energy. The most important contributions to kerma in C, N and O are from the 3α reactions in C, (n,α), (n,np) reactions in N and the (n,np) and 4α break-up reactions in O. A full description of the results is given in Dimbylow (1980).

A more complete approach to the calculation of reaction cross-sections requires the inclusion of discrete energy levels and precompound effects. This approach has been followed in the form of the multistep Hauser-Feshbach code, GNASH written by Arthur and Young [1]. The residual nuclei are composed of a set of discrete levels of known energy, spin and parity at low excitation energies joined to a continuum energy level density description at

the higher energies. The program uses a simplified pre-equilibrium expression which does not include spin and partiy effects, based upon the exciton model of Griffin[2]. The reaction cross-sections for Mg, Aℓ, P, S, Ar and Ca as well as for C, N and O have been calculated using GNASH.

Work is in progress to calculate the direct reaction components of the inelastic excitation of low lying energy levels in C and O which are produced by strong collective modes of excitation, using the Distorted Wave Born Approximation code DWUCK[3]. These are, in particular, the 2^+ level at 4.43 MeV and the 3^- level at 9.64 MeV in C and the 2+ (6.92 MeV), 3^- (6.13 MeV) levels in O. Direct components of (n, d) and (n, p) reactions are also being investigated.

Kerma values from the combined GNASH and DWUCK results will be calculated.

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DIMBYLOW, P.J., 1979 "The high energy neutron cross-section programs, NOPTIC and SMOLDERS", NRPB-M44.

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Title of project nr 2 : The measurement of stopping powers.

Scientific collaborators : Mr. A.A. Edwards, Mr. M.J. Whillock.

Objectives

In radiation protection the measurement of radiation dose is of great importance. Gas filled ionisation chambers provide the standard instrument by which other instruments and detectors are calibrated. To convert the ionisation current of a neutron irradiated ionisation chamber to dose rate in tissue the stopping powers of the charged particles created (protons, alpha-particles, carbon and oxygen ions) in both wall and gas material are required. There has been conflicting evidence with regard to the effect of phase (solid, liquid, gas) on stopping power, the most striking of which came from Williamson and Watt (1972) who presented data indicating that phase could have a 30% effect on stopping power for 1 MeV alpha-particles.

The objective of this contract was to verify the results given by Williamson and Watt and then to measure the phase effect for protons, carbon and oxygen ions at energies of relevance in radiation protection.

Method of Measurement and Results

Thin films of polyethylene were made and used to measure stopping power. Briefly, the mean energy of alpha-particles were measured both before and after passing through the thin film and from a measurement of the film thickness and the energy difference, stopping powers were derived. In the same apparatus the pressure of ethylene gas was adjusted to give the same thickness in $gm.cm^{-2}$ and the stopping power of the gas determined. Thus the mass stopping powers of ethylene and polyethylene were determined under the same conditions. Fig. 1 shows the experimental results obtained.

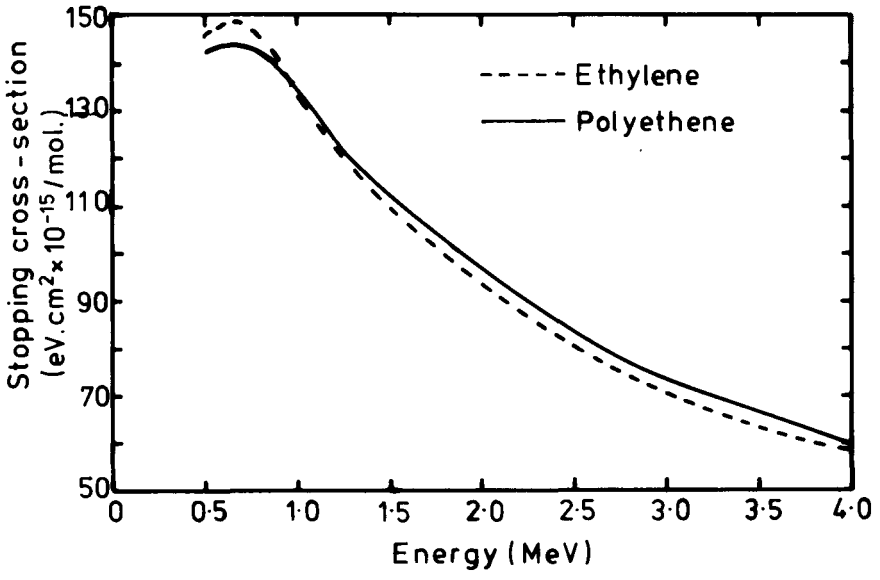

Figure 1. The stopping cross-sections of ethylene and polyethylene for α particles in the range 0.5 to 4 MeV.

The random errors associated with these measurements range from 0.5 to 2%. In addition the solid measurement may be systematically in error by an estimated standard error of 2% because of the random uncertainty attached to the measurement of film thickness. When comparing solid to gas measurements at any particular energy, 5% differences are not significant. At no energy between 0.5 MeV and 4 MeV is the difference between gaseous and solid stopping powers greater than this estimated uncertainty. At energies of 3 to 4 MeV the measured stopping power of the solid is about 5% higher than that measured for the gas. At 0.5 MeV it is 3% lower. This change of ratio is statistically significant showing that there exists a phase effect which varies with energy. The uncertainties are such that in region 3 to 4 MeV the gas is unlikely to have a stopping power greater than the solid. Comparing our results directly with those of Williamson and Watt the stopping powers for the gases agreed within about 5% but the results for the solid differed by between 20 and 30%. We concluded that the major cause of discrepancy between our conclusions and those of Williamson and Watt (1972) probably lies in the measurement of the thickness of solid films.

Having shown that the results of Williamson and Watt were not verified there seemed little virtue in measuring phase effects for protons, carbon ions and oxygen ions as originally planned. However, we noticed that our

measurements for the gas ethylene were generally larger than other workers, Williamson and Watt (1972), Bourland et al (1971), Palmer (1966). In an attempt to throw light on the cause of these differences which at some energies exceeded the quoted uncertainties, the stopping powers of other gases were measured with the same apparatus. These gases were N_2, H_2, CH_4, C_4H_{10} and C_3H_6. No completely consistent pattern emerged. The only other group who have measured the stopping power of all the gases we have measured are at Baylor University, Texas, Bourland et al (1971) and Lodhi and Powers (1974) and all results were obtained on the same apparatus. In the energy range 1-2 MeV our results are from 0% to 3% higher than the Powers group except measurements for propylene (C_3H_6) which are 4 to 7% higher and outside the quoted uncertainties. Checks on Bragg additivity using our data and that from the Baylor group failed to reveal which of the measurements was wrong. This discrepancy is still an outstanding problem. Details and a discussion of the measurements are to be found in Whillock and Edwards (1979).

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Title of project nr 3 : W-value measurements in gases of dosimetric importance.

Scientific collaborators : Mr. A.A. Edwards, Mr. M.J. Whillock.

Objectives

In the field of radiation protection it is essential to be able to determine dose, where the instrument used as a standard is the gas filled ionisation chamber. One of the parameters required to convert the current measured to the dose delivered, is the 'W' value of the gas used as the detecting medium. With this in mind a programme of work was undertaken to determine the W values of tissue equivalent gas and its constituents N_2, CH_4 and CO_2 using alpha particles in the range 1 - 4 MeV.

Method of Measurement and Results

An ionization chamber was designed based on the co-axial system with charge collection on the central electrode. The chamber is used in two different modes:

a) a fast pulse counting system using an amplifier and scaler to determine the number of alpha particles entering the chamber in a given period,

b) a total charge accumulating device using a vibrating reed electrometer over an identical period.

The energy of the incident particles is determined by means of a surface barrier detector. A natural alpha emitter (Pu^{238}) is the beam source, the energy of which is varied by means of control absorbers positioned between the source and the entry window of the chamber.

Measurements using N_2 and CH_4 have been made at five energies in the range 1 - 4 MeV, with both sets of results indicating that the W value is proportional to the reciprocal of the beam energy.

The random errors are 0.5% or better. The results are consistently higher than those found by other workers (Valma and Baum 1978; Jesse 1961). For CH_4 a comparison with the results of Valma et al (1978) shows differences ranging from 2% (at about 4 MeV) to 5% (at 1.1 MeV), whereas for N_2 the difference falls with decreasing energy from 3% to 1%. However, a comparison of the present work with Jesse's (1961) results for N_2 shows a

difference which increases with decreasing energy, going from 2% to 3%. The reasons for these discrepancies among these reported values is yet to be resolved. Currently measurements are being made using CO_2 and these will be followed by similar work using Rossi-type tissue equivalent gas.

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Title of project nr 4	:	Solid state physics processes underlying the properties of some materials used in thermoluminessence dosimetry.
Scientific collaborators	:	Dr. J.A. Reissland Dr. A.F. McKinlay, Dr. C.M.H. Driscoll and Dr. D.T. Bartlett.

1. Introduction

The main objective of the research programme has been to study the physical processes underlying the properties of materials used in solid state dosimetry, with a view to improving existing methods of dosimetry and developing new techniques for specific applications, including mixed field dosimetry and environmental monitoring. The main techniques studied have been thermoluminescence and lyoluminescence. Thermally stimulated conductivity initially considered feasible for fast neutron dosimetry has proved disappointing due to inadequate sensitivity.

2. Thermoluminescence Dosimetry Research

Significant progress has been made in the following areas of thermoluminescence research:

(1) The effects of LET on the thermoluminescence responses and light conversion efficiencies of lithium fluoride and lithium borate.

(2) The development of the ultra violet phototransfer technique in lithium fluoride and its application to the routine re-assessment of absorbed dose in LiF:PTFE elements.

(3) Factors affecting the background and sensitivity of thermoluminescence phosphors.

(4) The interpretation of peaks between 200^{o} and 350^{o}C in LiF.

(5) The characterisation of the thermoluminescence properties of new phosphor preparations with acceptable photon energy tissue equivalence and good sensitivity for biological and medical applications.

(6) The development of an environmental gamma dosemeter based on LiF:Mg:Ti and CaF_2:Dy thermoluminescent elements.

(7) The development of thermoluminescent dosemeter elements for surface and skin dosimetry.

LET effects (Charged Particle Response)

The responses and relative light conversion efficiencies have been established for lithium fluoride (TLD 700) irradiated with alpha particles of mean energies 2.4, 4.3, 5 and 5.7 MeV, protons, and helium-3 ions of 10 MeV per a.m.u., and ^{60}Co gamma radiation, for absorbed doses up to 10^5 Gy [6,8]. The results show a general correspondence between the thermoluminescence per unit energy deposited and LET, and the thermoluminescence per unit energy deposited and absorbed dose of ^{60}Co gamma radiation. The charged particle responses have been analysed on the basis of the track structure approach of Katz. The response of lithium borate ($Li_2B_4O_7$:Mn, British Nuclear Fuels Ltd.) irradiated with 2.4 and 5.7 MeV alpha particles has also been established [12]. The ratio of the thermoluminescence response of peak 6 (285°C) to peak 5 (210°C) in lithium fluoride increases with LET [14].

The differential thermoluminescence response of thin lithium fluoride polyimide discs to alpha particles and gamma radiation was measured to assess their suitability for mixed field dosimetry. Over the absorbed dose range 10^{-2} to 3 Gy, the ratio of the response of peak 6 to peak 5 was approximately seven times higher for high LET radiation (120 keV μm^{-1}) than for low LET radiation (0.3 keV μm^{-1}). The dosemeters are potentially useful for mixed field dosimetry where the LET components of the mixed field are known.

Environmental and other effects affecting the stability of dosemeters

In addition to the LET response studies described previously, an investigation has been completed on the effects of environmental factors on the relative sensitivity of peaks 5 and 6 [15]. This has led to the postulation of a model of the peak's behaviour [16] which will be tested further by optical absorption and luminescence studies. The effects of grain size, thermal treatment, atmospheric environment during storage, visible and ultra violet radiation, and surface cleaning procedures, on the background response ('zero' absorbed dose response) of lithium fluoride powder and PTFE based discs have all been studied [15,16, 17].

The main conclusions resulting from these studies are:

(1) The most commonly observed background effect is due to energetically deep trapping centres, associated with glow peaks at temperatures between 280 and 300°C, which are not emptied by a 300°C anneal. This can result in the leading edges of these high temperature peaks being read out.

(2) The mean grain size of lithium fluoride powders progressively reduces following successive high temperature (300-400°C) anneals.

(3) The thermoluminescence sensitivity of powder decreases with decreasing grain size.

(4) The background response under normal environmental storage over a period of several months increases with decreasing grain size for peak 6, but decreases for peak 5.

(5) The background signal of LiF:PTFE discs can be reduced by 90% by surface cleaning in a solution of hydrochloric acid and methanol.

(6) Storage in an atmosphere containing oxygen in concentrations exceeding 50,000 ppm increases the background response.

Phosphor performance and applications studies

New phosphor preparations of established dosemeter materials (eg. LiF and $Li_2B_4O_7$) have been studied with a view to finding a high sensitivity phosphor with approximate photon energy tissue equivalence. High sensitivity dosemeters are also required in the development of environmental dosemeters. A three element dosemeter, comprising LiF (TLD 700) and two CaF_2:Dy (TLD 200) chips, is being assessed for the measurement of the gamma component of the radiation from uranium decay products incorporated in building materials. Two elements measure the environmental absorbed dose and provide photon energy discrimination. The third element is predosed and measures thermal fading - $CaSO_4$-Dy (TLD 900) has also been studied as an alternative to TLD 200.

Re-assessment of Absorbed Dose

It has been found that the Mayhugh-Fullerton technique of sensitization of lithium fluoride was incompatible with the re-assessment of absorbed doses less than 0.5 Gy using the ultra violet phototransfer thermoluminescence technique[11]. An anneal regimen of 150 hours at 300°C has proved successful in the reduction of interference effects due to previous dose history and exposure to ultra violet radiation. The technique of re-assessment of absorbed dose has been introduced as part of the NRPB automated thermoluminescence dosimetry service [10,18]. A semi-automated re-assessment module has been built.

3. Lyoluminescence Research

Apparatus was designed and constructed for the study of lyoluminescence. Photon counting techniques were used enabling accurate and reproducible measurements over a large range of absorbed dose with excellent stability of detection and amplification. Electron spin resonance investigations of free radical formation in polycrystalline mannose and glucose monohydrate established the characteristics of the spectra and the radical yield as a function of absorbed dose and pre- and post-irradiation treatment. It was concluded that free radicals produced in the bulk solid during irradiation are the principal agents of lyoluminescence via the formation of peroxyl radicals on dissolution. Absorbed dose response characteristics have been established as a function of different pre- and post-irradiation treatments. Post-irradiation storage of mannose produces an enhancement of response at high levels of absorbed dose and a loss of supralinearity at low levels. Pre-irradiation annealing at 100°C gives rise to an overall increase in response. The absorbed dose responses of mannose and glucose monohydrate to 2.4 and 5.65 MeV alpha particles and Helium-3 (10 MeV per amu) were measured. Comparisons were made of the responses of lyoluminescent dosemeters and ionization chambers as a function of depth dose in a π^- meson beam [1-9].

4. Conclusions

In summary, the solid state dosimetry programme has provided original and relevant information on many factors affecting dosemeter performance important to all areas of ionizing radiation dosimetry as well as to an understanding of the fundamental processes of thermo and lyoluminescence.

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Contractant de la Commission :

COMMISSARIAT A L'ENERGIE ATOMIQUE

N° du contrat : 177-76-1 BIO F

Chef du Groupe de Recherche : Dr. N. PARMENTIER

Thème général du contrat : HEAVY CHARGED PARTICLE DOSIMETRY

Titre du projet : DOSIMETRIE DES PARTICULES LOURDES CHARGEES

Chef de projet et collaborateurs scientifiques : Dr. N. PARMENTIER
M. CHEMTOB
V.D. NGUYEN

I. ETUDES MENEES AUPRES DU FAISCEAU DE 650 MeV (SATURNE I) -

V.D. NGUYEN, P. FACHE, M. CHEMTOB.

L'objectif des expériences commencées en 1975 était de préparer un faisceau pour la radiothérapie. Pour ce faire, les études suivantes ont été entreprises.

1 - Etudes dosimétriques du faisceau de 650 MeV

Ces études ont nécessité la mise au point et la construction de plusieurs chambres d'ionisation, de géométrie différente (plane à faces parallèles et cylindrique) [1]. Elles ont commencé par le tracé du pic de Bragg (rapport pic sur entrée 4,25 avec un collimateur de 7 cm de diamètre). Elles se sont poursuivies par l'installation d'un dégradeur isodose, donnant un plateau de dose de 5 cm de profondeur dans l'eau. La pente de ce plateau était de 2 % par centimètre de profondeur (rapport plateau sur entrée 1,7 en moyenne). Ce dégradeur était constitué de quatre secteurs. Chaque secteur comportait 9 épaisseurs de cuivre et tournait plusieurs fois par impulsion de SATURNE [2]. Compte tenu des résultats de microdosimétrie et de radiobiologie menées parallèlement, un essai a été tenté pour construire un plateau de dégradeur isoeffet.

2 - Etudes microdosimétriques

Ces études avaient pour but de mesurer la variation de $\bar{y}_D$ en fonction de la profondeur de parcours des hélions de 650 MeV. Au début du parcours,

$\bar{y}_D$ est égal à 4,5 keV.μm^{-1}, passant par une valeur constante égale à 9,3 keV. m^{-1} sur le plateau du dégradeur isodose (sur environ 3 cm) et atteignant 22 keV.μm^{-1} (à la fin de ce même plateau) (2).

3 - Etudes radiobiologiques

Plusieurs effets biologiques ont été étudiés :

1)- Etude des aberrations chromosomiques dans les lymphocytes humains (DPr/SRTE)

2)- Etude de la survie des cellules tumorales EMT.6 (INSTITUT Gustave ROUSSY, Service de Radiobiologie Clinique).

Ces échantillons ont été irradiés à la profondeur pour laquelle $\bar{y}_D$ est égal à 9,3 keV.μm^{-1}. (3).

Pour les doses faibles délivrées par les hélions, il est apparu que l'E.B.R. des effets biologiques précités était proportionnel à ξ_α / ξ_γ, ce qui nous a conduit à étudier un dégradeur permettant d'obtenir un plateau isoeffet, en tenant compte des variations de $\bar{y}_D$ en fonction de la profondeur donnée, au paragraphe précédent. Un autre dégradeur a été construit en pondérant chaque profondeur par le $\bar{y}_D$ correspondant.

Les mesures de microdosimétrie faites avec ce dégradeur à 5,81 g.cm^{-2} de profondeur (entrée dans le milieu) et à 15,21 g.cm^{-2} avant la fin du parcours (17,82 g.cm^{-2}) donnent respectivement les valeurs $\bar{y}_D$ de 3 keV.μm^{-1} et de 6,3 keV.μm^{-1}. Le gain dans la réalisation du plateau isoeffet a été considérable mais encore insuffisant. La transformation de SATURNE I, en 1977, a arrêté cette étude.

4 - Etudes dosimétriques et microdosimétriques sur SATURNE II

Pour l'étude des effets à long terme des rayonnements (tentative de compréhension des mécanismes de radiocarcinogénèse), ce type de faisceau présente l'avantage de pouvoir irradier les échantillons biologiques et les animaux avec une gamme étendue de valeurs du transfert linéique. Ils présentent de plus un intérêt balistique permettant d'irradier avec précision le volume d'intérêt. Pour profiter au mieux de ces qualités, il est nécessaire de disposer d'un système de mise en place parfaitement reproductible et d'exploiter les résultats de mesure par un calculateur en ligne. C'est ce qui a fait l'objet du travail en 1979 et 1980 (4).

Deux types de montage sont utilisés :

- un appareil télécommandé permettant de faire varier l'épaisseur d'eau traversée par les particules ;
- une cuve avec explorateur de fantôme télécommandé du type utilisé en radiothérapie.

Le contrôle du faisceau est assuré par une chambre à fils et un ensemble de chambres d'ionisation.

La détermination de la dose au niveau de l'échantillon biologique ou de l'animal est obtenue à l'aide de la chambre à transmission (M1) et d'une chambre de type crayon placée à côté de l'échantillon biologique et servant de moniteur secondaire.

L'établissement de la cartographie du faisceau est fait à l'aide d'une chambre mosaïque plane, à faces parallèles en plastique équivalent au tissu, comportant 61 volumes élémentaires de mesure.

Les programmes de calcul actuellement disponibles permettent :

- d'obtenir directement, à partir des lectures brutes, les doses en rad normalisées par rapport à la chambre (M1) de manière séquentielle pour chaque déplacement ;
- de calculer immédiatement la dose absorbée au niveau de l'échantillon biologique irradié.

L'ensemble a été testé lors de deux séries d'expériences à SATURNE II, avec des faisceaux d'hélions de 487, 554 et 654 MeV.

II. ETUDES EXPERIMENTALE ET THEORIQUE DES GRANDEURS DE BASE POUR LA DOSIMETRIE DES PARTICULES CHARGEES LOURDES

L'énergie correspondant à la création d'une paire d'ions ($\bar{W}$) et le pouvoir d'arrêt des particules chargées (S) présentent un intérêt fondamental en dosimétrie des champs mixtes. En effet, actuellement, les techniques utilisant la méthode des chambres d'ionisation doubles, complétée par le compteur G.M. et le film sont considérées comme les plus précises et les plus directes pour déterminer les doses dues aux neutrons et aux gamma, dans un champ mixte. Les mesures obtenues par ces techniques sont corrigées par le rapport $\bar{W}_C / \bar{W}_N$ (valeurs de $\bar{W}$ lors de la calibration gamma, et lors de la mesure aux neutrons) et par le rapport $(S_{m,g})_C / (S_{m,g})_N$ (valeurs des rapports des pouvoirs d'arrêt entre le milieu et le gaz à l'étalonnage gamma au même rapport, lors de la mesure aux neutrons).

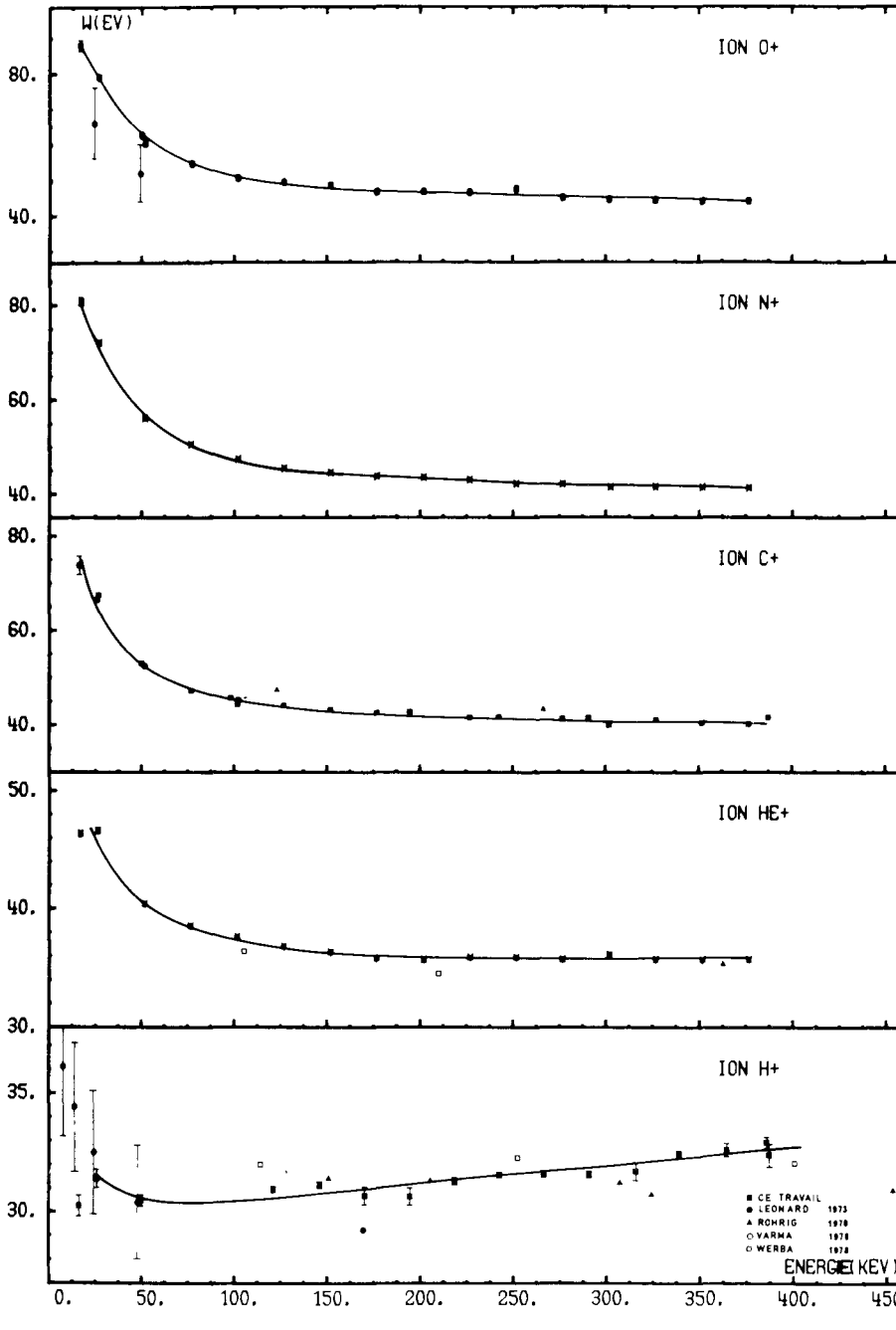

Fig. 1 VARIATION DE $\bar{W}$ AVEC L'ENERGIE DES IONS INCIDENTS DANS LE GAZ ET Fig. 1

Elles permettent d'étalonner les autres dosimètres, qu'ils soient utilisés en radiothérapie, radiobiologie ou radioprotection.

L'objectif des mesures de $\overline{W}$ et de S pour les particules chargées est donc de mieux préciser les valeurs à prendre pour l'interprétation en terme de doses des mesures ionométriques.

1 - Etudes expérimentales de $\overline{W}$ (E) (V.D. NGUYEN, J. CHARY, F. POSNY)

La mesure de cette grandeur en fonction du type d'ion incident, de son énergie, dans différents gaz (CH_4, CO_2, N_2, T.E., et mélanges gazeux) a été faite d'une manière très détaillée. Les résultats obtenus ont fait l'objet de nombreuses publications (5, 6, 7, 8). A titre d'exemple, les résultats sont donnés pour le gaz E.T. de Rossi (Figure 1). Il a été constaté pour les ions H^+ pénétrant et s'arrêtant dans tous les gaz précités, que $\overline{W}$ passait par un minimum pouvant s'expliquer par un phénomène d'échange de charge, donc d'une diminution de l'ionisation dans un domaine d'énergie compris entre 25 et 300 keV.

2 - Etudes théoriques de $\overline{W}$ (E) (M. CHEMTOB, V.D. NGUYEN, P. GOUARD)

Cette étude est un cas particulier du ralentissement des particules chargées dans les gaz. En conséquence, le travail a débordé largement l'étude de $\overline{W}$. Il a été nécessaire d'étudier les pouvoirs d'arrêt. A titre d'exemple, les pouvoirs d'arrêt sont donnés sur les figures 2 et 3. Puis l'utilisation du programme de Winterbon a permis d'obtenir les tables de parcours des particules chargées de basse énergie dans les gaz dosimétriques, des tables de parcours où l'ionisation a lieu, et des valeurs théoriques des rapports $\overline{W}(E) / \overline{W}_\infty$. Une thèse a été présentée à l'Université de PARIS VII, donnant pour les gaz monoatomiques et diatomiques les résultats des variables précitées, dans la gamme d'énergie 1 à 500 keV (9).

3 - Etudes du parcours et du pouvoir d'arrêt des ions lourds dans les gaz (M. CHEMTOB, F. FERNANDEZ, J. CHARY).

Les études du parcours d'ionisation extrapolé sont faites pour les ions H^+, He^+, C^+ dans les gaz purs (CO_2, CH_4, N_2) et dans différents mélanges, notamment le gaz E.T.. Le montage expérimental est constitué essentiellement :

- d'une enceinte cylindrique remplie du gaz à étudier,
- d'une chambre plane à faces parallèles comportant deux grilles, et d'une

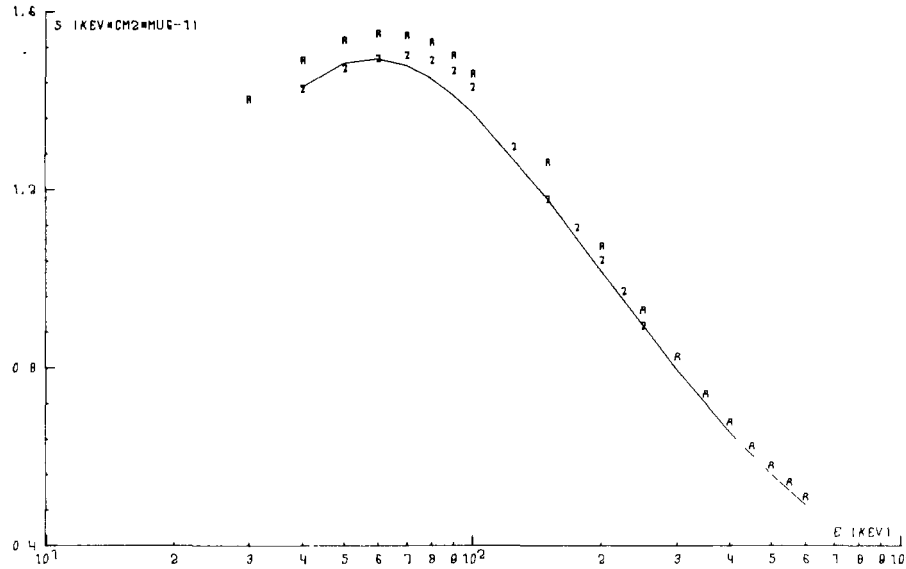

Fig.2 POUVOIR D'ARRET ELECTRONIQUE (H DANS CH4)

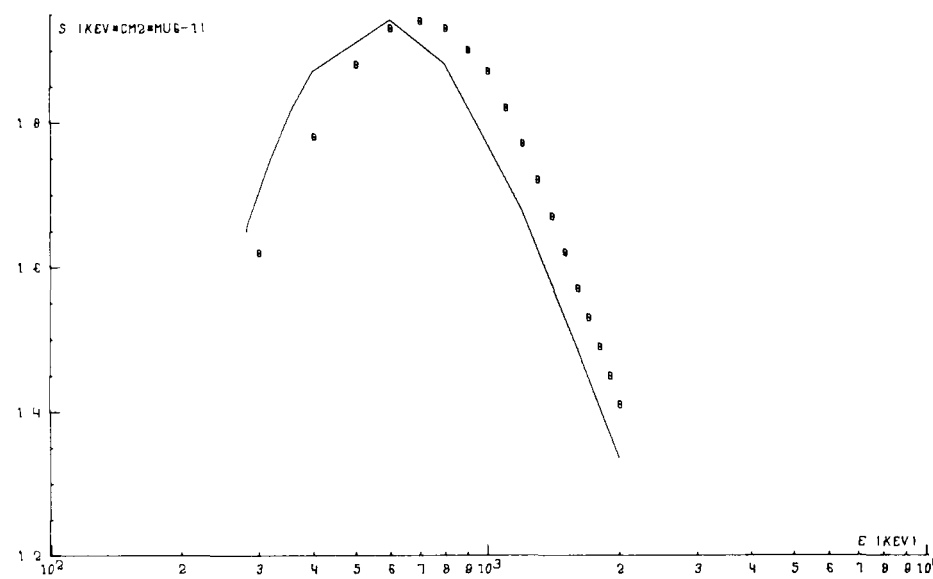

Fig 3 -POUVOIR D'ARRET ELECTRONIQUE (HE DANS CO2)

plaque collectrice. Cette chambre se meut perpendiculairement à l'axe du faisceau.

Du fait qu'en fin de parcours des ions les chocs élastiques prédominent, le parcours d'ionisation extrapolé diffère du parcours des particules incidentes. Cette différence est faible pour les ions H^+ et He^+ dans la gamme 50 keV. Elle est, par contre, notable pour les ions les plus lourds. La dérivation du pouvoir d'arrêt électronique à partir du parcours d'ionisation extrapolé n'est donc possible que dans le cas des protons (Figure 4) et des alpha. Les résultats ont fait l'objet de deux publications en 1980 [10, 11].

Sur le même montage a été installée une chambre multiple (48 fils) pour la mesure des profils latéraux d'ionisation, des particules subissant un ralentissement en profondeur dans le gaz méthane utilisé comme test.

Cette expérience est au stade de la mise au point.

Des études plus approfondies sont nécessaires pour la mesure du pouvoir d'arrêt des particules plus lourdes que les ions He^+ entraînant un changement de montage. Ce montage est au stade de la préétude.

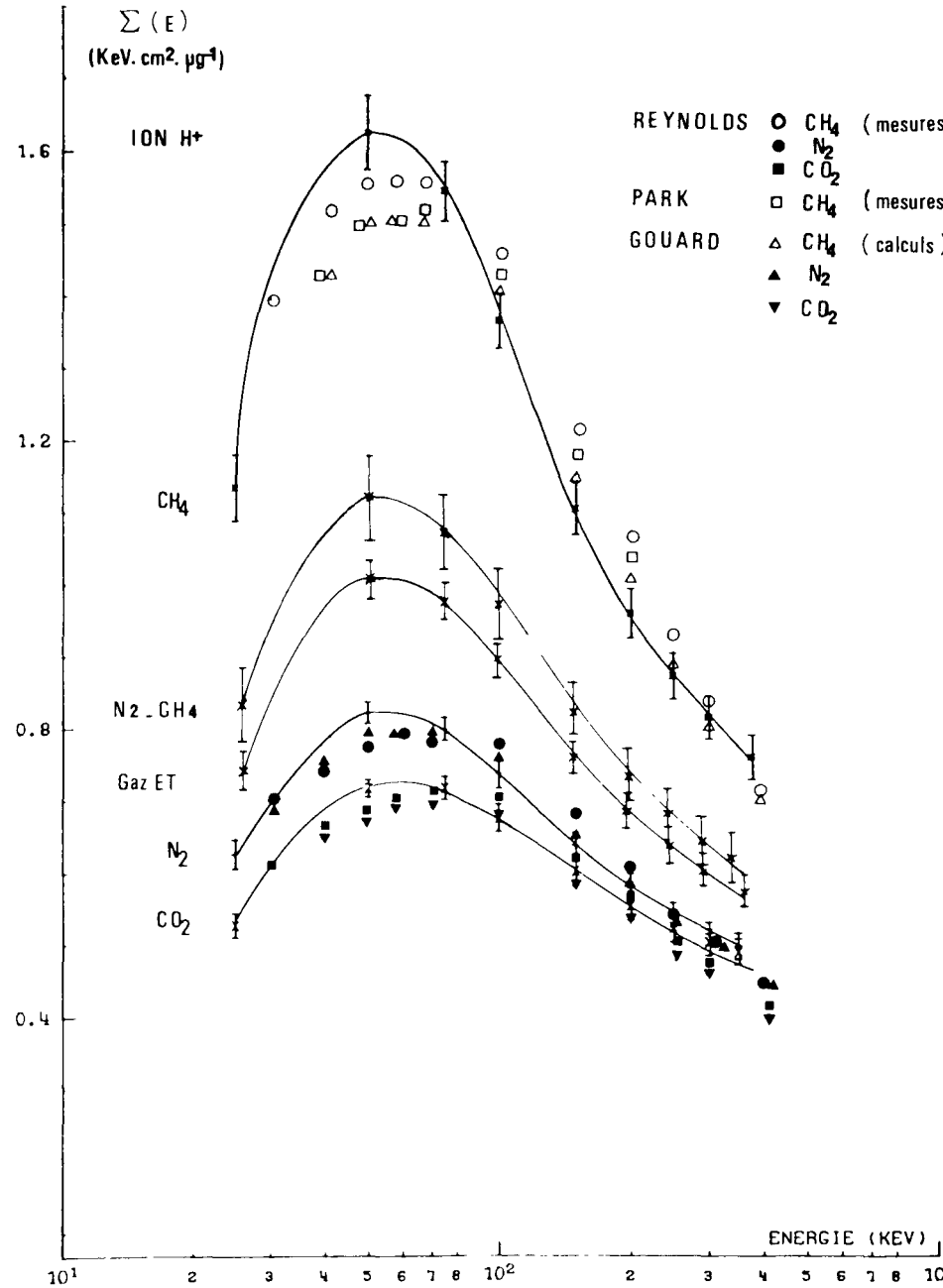

Fig. 4 PERTE D'ENERGIE PAR UNITE D'EPAISSEUR TRAVERSEE

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Nr. des Vertrags: 215-76-10 BIO D
Leiter der Forschungsgruppe(n): Prof.Dr.med. L.E. Feinendegen

Allgemeines Thema des Vertrags: Biological Effects of Incorporated Isotopes and Radiation of High Ionization Density

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Titel des Projekts Nr. 1: Microdosimetric Studies on the Biological Effectiveness of Incorporated Radioactive Nuclei

Leiter des Projekts und wissenschaftliche Mitarbeiter:
J. Booz, L.E. Feinendegen, H.D. Hartfiel, J. L. Humm,
G. Tisljar-Lentulis, Th. Smit
and D. E. Charlton (Concordia University, Montreal)

It was the aim of the investigations to improve our understanding of the radiobiological effects of incorporated radioactive nuclides in comparison to the effectiveness of external radiations. The radionuclides investigated were ^{3}H, ^{125}I, ^{123}I, ^{64}Cu and ^{57}Fe, i.e. emitters of Auger- and other low energy electrons that are used in biological radiodiagnostic experiments. The central point of the research studies was the question as to the relative contribution of the transmutation effects of these radionuclides, when incorporated into the DNA of mammalian cells, as compared to the local radiation effects of the low energy electrons emitted. In pursuing these aims, in close collaboration with the corresponding biological investigations described under project 2, it was necessary to consider in detail the atomic electron transitions following the disintegration which give rise to complex number and energy distributions of the emitted electrons. These results led to more precise dosimetric data for radioactive nuclides specifically incorporated in DNA. They also led to the establishment of a model on the biological effectiveness of radionuclides in the DNA considering both the transmutation effect and the radiation effect of the emitted electrons. The results have contributed to a better understanding of the biological effectiveness and radiation mechanisms of incorporated radionuclides and have improved the dosimetric basis of radiation risk calculations of those radionuclides.

Survey on the results in detail:

Calculation of the number and energy distributions of electrons emitted per disintegration of 125-I, 123-I, 64-Cu and 57-Fe in condensed medium. Influence of the chemical phase of the matter for disintegrations of 125-I (condensed medium, isolated atom, gasions CH_3I). Monte Carlo simulation of the track structure in water produced by disintegrations of 125-I and its emitted electrons. Calculation of the mean energy deposition per disintegration of 3-H and 125-I homogeneously distributed in volumes of 1 nm to 100 µm, including information on the standard deviation of the mean energy deposition. Development of a model of the biological effects of radionuclides incorporated into DNA. In this model the number of emitted electrons, i.e. the number of positive charges remaining on the DNA, is related to the transmutation effect whilst the energy imparted to the DNA by the emitted electrons is related to the electron dose effect. Clarification of the relation between the decreasing slope of mammalian-cell survival curves after radionuclide incorporation in DNA and non-homogeneous incorporation. Measurement of electron ranges and W-values in different gases for energies of 20 eV to 2 keV. Calculation of the energy spectrum of primary electrons produced by different photon radiations. Calculations of the number and energy distributions of Auger electrons induced by photons below and above the K-absorption edge.

For iodine-123, iodine-125 and Cu-64 the probability of disintegrations as a function of both the number and the total energy of the electrons emitted was calculated. An example is shown in Fig. 1. Disintegrations of iodine-125 and the tracks of the electrons emitted were simulated by Monte Carlo calculations in collaboration with Mr. Paretzke, Neuherberg (Fig. 2). Results on the mean electron number and on the mean energy emitted by electrons and photons per decay are given in Table I for different radionuclides. Tritium is shown for comparison.

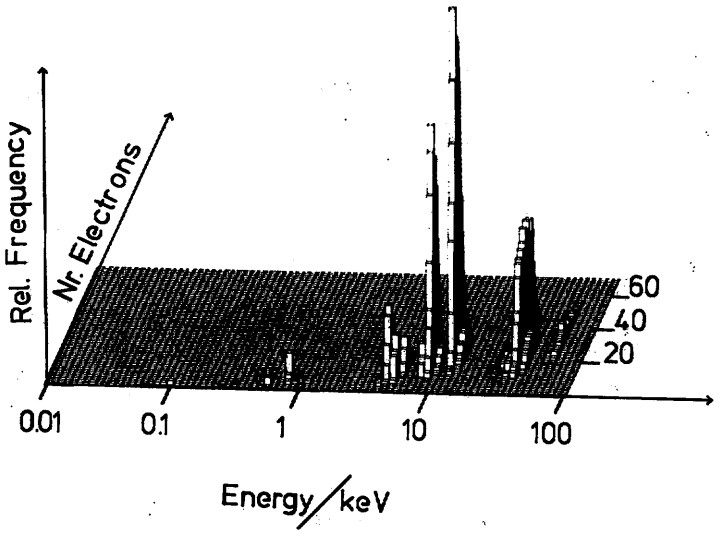

Fig. 1: Threedimensional plot of the probability of 125-I disintegration as a function of both the number and the total energy of emitted electrons.

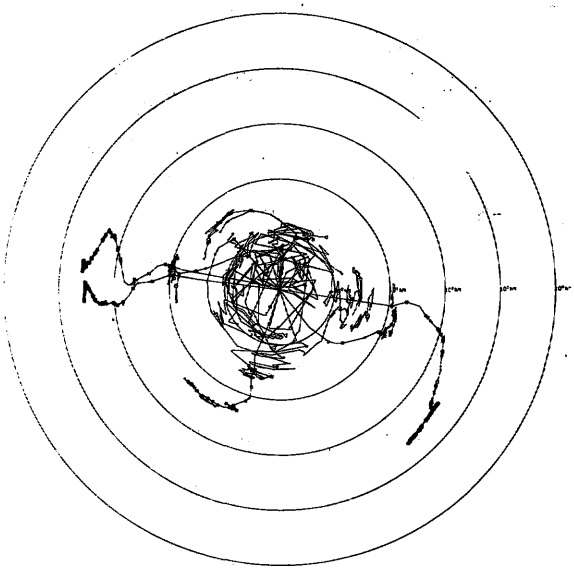

Fig. 2: Computer simulated electron tracks of electrons emitted by a typical computer simulated disintegration of 125-iodine. 20 electrons are emitted carrying away 12.2 keV in total. Squares - Ionization; crosses - excitations. (Calculation performed in collaboration with H. Paretzke, Neuherberg).

Table I

Number of electrons, $\bar{n}$, energy of electrons, E_{el}, and energy of photons, E_{ph}, per disintegration

	125-I	123-I	64-Cu	57-Fe	3-H
$\bar{n}$	21.1	12.3	2.2	4.7	1.0
E_{el}/keV	19.9	28.8	179.9	4.13	5.73
E_{ph}/keV	41.6	167.3	10.8	1.64	-

In phosphorous, X rays of energies immediatly below and above the K-absorption edge liberate 2.4 and 4.0 electrons respectively. For bromium the corresponding numbers of electrons are 5.8 and 6.7

Monte Carlo calculations were performed on the primary electron spectra of 10 keV photons, of X rays of 105 kVP, and of iodine-125. In addition the spectra of potential Crosser-Insider-Starter-Stopper electrons were calculated, i.e. spectra of those electrons which had a sufficiently high probability of depositing some or all of their energy in a sensitive volume as crossers, insiders, starters or stoppers.

Titel des Projekts Nr. 2: Biological Consequences of Heterogeneous Dose Distributions from Incorporated Nuclides Others than Bone Seekers

Leiter des Projekts und wissenschaftliche Mitarbeiter:
G. Tisljar-Lentulis, L.E. Feinendegen, P. Henneberg, Th. Mielke, F. Schneeweiß

This project investigated the biological aspects of the same central problem that was tackled in project 1, namely the relation between the transmutation effect and the radiation dose effect of radionuclides incorporated into the DNA of mammalian cells. The implications of this problem for radiation protection lie in the significance of mutations for long term effects. It is well known and it has been shown by the results of this project that mutations and single strand breaks may be drastically increased by disintegrations in the DNA, e.g. of tritium. On the other hand cell survival, the significance of which for radiation protection is frequently overestimated, does not show a significant influence from transmutation effects. The end points investigated in this project were single- and double-strand break of isolated DNA and after irradiation of intact cells in culture, and cell survival. Tritium and 125-iodine was incorporated into DNA via 3H-TdR, 3H-IUdR and 125-IUdR. Results were obtained for RBE relative to 60-Co gamma rays, OER and repair.

Survey on the results in detail:

Measurement of single-strand breaks after incorporation of 125-I into DNA of human-kidney T cells, in deep frozen state: 4.1 single-strand breaks per disintegration in intact cells, repair of 70 - 80%; in isolated DNA 2.0 single-strand breaks. Measurement of double-strand breaks after incorporation of 125-I into DNA of human kidney T cells, disintegration in intact cells: at least 1 double-strand break per disintegration, no repair. Measurement of single-strand breaks after incorporation of 3H-TdR and 3H-IUdR. Exposure of intact cells in deep frozen state: 3H-TdR - 0.9 to 1.1 breaks per disintegration, repair 80 - 85%; 3H-IUdR - 1.1 to 1.3 breaks per disintegration, repair 70 - 85%. Measurement of double-strand breaks after incorporation of

3H-TdR and 3H-IUdR. Exposure of intact cells in deep frozen state: 3H-TdR-0.12 breaks per disintegration. repair 35 - 40%. 3H-IUdR - 0.14 breaks per disintegration, repair 10 - 20%. Survival curves after incorporation of 125-I showed an exponential slope with a D_{37} of 135 disintegrations under aerobic conditions. Results on OER after incorporation of 3H-TdR, 3H-IUdR and 125-IUdR for single- and double-strand breaks (exposure of intact cells at 0 - 1°C) and cell survival (exposure at 4°C) are given in table I. For single-strand breaks from 3H-TdR the transmutation effect contributes with about 50% to the effect. For double-strand breaks and survival after disintegration of 3-H in DNA as well as for all end points after disintegration of 125-I in DNA there is no significant transmutation effect.

Table I: OER for different end points

60-Co rays	Single-strand breaks	4.5
	Survival	2.2
3H-TdR	Single-strand breaks	1.6
	Double-strand breaks	3.9
	Survival	2.8
3H-IUdR	Survival	2.2
125-IUdR	Single-strand breaks	1.2
	Double-strand breaks	1.4
	Survival	1.0

he low OER of 1.6 for single-strand breaks after tritium isintegration in the DNA was interpreted as the result of he transmutation effect of tritium, because this effect hould not depend on the presence or absence of oxygen. ased on this consideration the contribution of the trans- utation effect, $t/(E_{N_2} + t)$, was calculated with:

$$OER_{^3H} = \frac{E_{N_2} \times OER_{\gamma} + t}{E_{N_2} + t}$$

Titel des Projekts Nr. 3: Microdosimetric Studies on the Biological Effectiveness of Directly and Indirectly Ionizing Radiations.

Leiter des Projekts und wissenschaftliche Mitarbeiter:

J. Booz, B. Bauer, L.E. Feinendegen, J. Fidorra, Th. Mielke, A. Poli, Th. Smit

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The evaluation of radiation quality and of the mean quality factor of mixed neutron-gamma rays are fundamental prerequisits for the assessment of radiation risk in practical radiation protection and in neutron therapy. This is a twofold problem. On the one hand it is important to know the real relationship between absorbed dose, radiation quality and effects at low doses; on the other hand it is essential to have a practical description of radiation quality which can be easily measured and which allows for a reasonable estimate of the effect. In this project both aspects have been investigated, however, the second one, the practical aspect, was considered as more important.

On the fundamental side, microdosimetric spectra were measured for a large variety of gamma- and neutron radiations, free in air and in a water phantom. A biparametric definition of radiation quality was introduced, a plot of $\overline{y_D}$ against $\overline{y_F}$, which allows for a clear separation of low LET, high LET, and mixed radiations, and also for a specification of the radiation quality of a particular radiation within the three groups. Here $\overline{y_F}$ is the frequency mean lineal energy and $\overline{y_D}$, the dose mean lineal energy, reflects the fluctuation of $\overline{y_F}$. Regions of low, medium and high dose were defined by comparing the magnitude of absorbed dose with both the frequency and dose mean specific energies, i.e. microdosimetric parameters which are proportional to respectively $\overline{y_F}$ and $\overline{y_D}$. Survival of mammalian cells was measured for different mixed n-γ radiations, and it was shown that in the microdosimetric spectra the changes with radiation quality are much stronger than the corresponding changes in RBE.

On the practical side, it was shown that the parameter y*, the $\overline{y_D}$ corrected for saturation where overkill is observed for the given system, is proportional to the initial slope of survival curves for a large range of neutron energies (0.1 to 15 MeV). Therefore y* was recommended and employed as a practical radiation quality estimate for the evaluation of neutron RBE at low doses. In this way it was shown that in a water phantom the low dose neutron RBE does not change significantly with depth or with lateral displacement so that in neutron therapy one can work with a constant neutron RBE. For the purpose of evaluating the mean quality factor of mixed n-γ radiations, the four-component method was developed. This method allows to evaluate $\bar{Q}$ from the microdosimetric spectrum in such a way that the ICRP recommendations are respected. Details of this method are described below.

Survey on the results in detail:

The problem of the measurement and the description of radiation quality of mixed neutron-gamma fields was the central point of investigation of this project in the last years. The most important subject was the development of a method for the determination of the mean quality factor, $\bar{Q}$, of mixed n-γ rays from microdosimetric spectra. These efforts resulted in the establishment of the four-component method which separates microdosimetric spectra into the contributions of γ rays, fast recoil protons, slow recoil protons, and heavier recoil ions. Due to this separation the method considers the ICRP recommendations on Q(LET) very closely and also allows for conversion from measured ionization to energy imparted.

The separation of the four particle components in microdosimetric spectra of mixed n-γ radiations is shown in Fig. 1. Once the dose fractions and the $\overline{y_D}$ of the four components are known, the total mean quality factor is calculated as the sum of the $\bar{Q}$ of the four components weighted by the dose fractions and by the dose mean LET, $\overline{L_D}$. It is the advantage of the four-

component method that specific relations between $\overline{y_D}$ and $\overline{L_D}$ can be used which respect recommendations of ICRP: γ - rays: Q = 1, irrespective of $\overline{y_D}$; fast recoil protons: $\overline{y_D}$ = (9/() $\overline{L_D}$; slow recoil protons: $\overline{y_D} = \overline{L_D}$; heavy recoil ions: Q = 20, irrespective of $\overline{y_D}$. For both types of recoil protons a linear relationship between Q and L is used: $Q = 1 + L_\infty/6$.

The dose contribution of these four components as a function of lateral distance from the beam axis is given in Fig. 2.

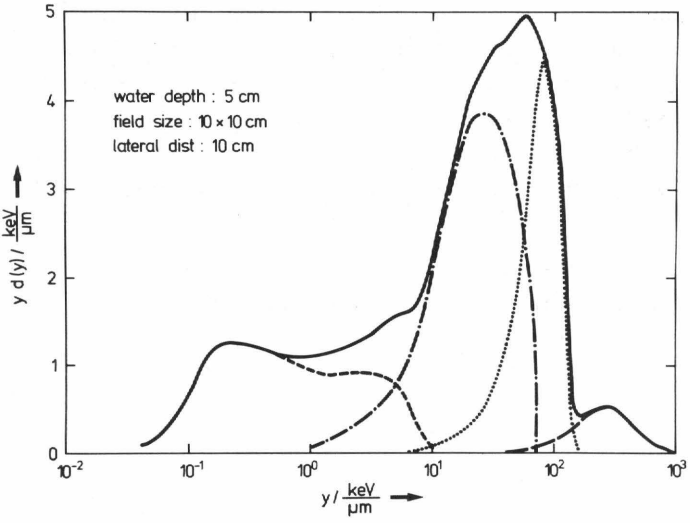

Fig. 1: Distribution y d(y) at 5 cm water depth and at a lateral distance of 10 cm from the beam boundary of a collimated fast neutron beam. Separation into the four radiation components: gamma rays, fast recoil protons, slow recoil protons and heavier recoil ions (from left to right). $y^* = 24$ keV/µm.

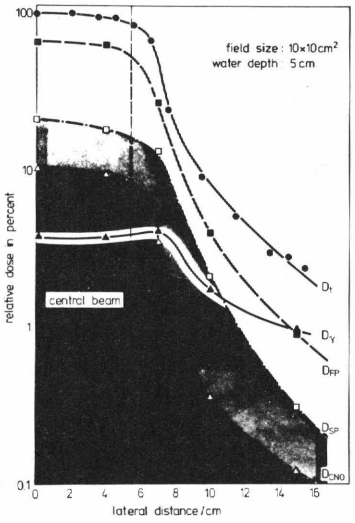

Fig. 2: Dose-profile curves of the four radiation components at 5 cm water depth: D_t - total absorbed dose; D_{FP} - dose fraction of fast recoil protons; D_{SP} - dose fraction of slow recoil protons; D_{CNO} - dose fraction of heavier recoil ions; D_γ - gamma dose fraction.

Titel des Projekts Nr. 4: In Vivo Studies of Biochemical Reaction Rates from Low Dose Irradiation

Leiter des Projekts und wissenschaftliche Mitarbeiter:
W. Porschen, H. Mühlensiepen, J. Booz, L.E.Feinendegen

It was the objective of this project to investigate the challenging phenomenon of suppression of IUdR incorporation in cells due to irradiation of mice with low and very low doses (0.5 to 100 rad). The final aim is the development of a biological accident dosimeter. In 1976-80 the dependence of this hypersensitive in vivo effect was analyzed with regard to different experimental methods and environmental conditions. The highest low-dose sensitivity was found when the capability of IUdR incorporation of cells from irradiated mice was measured in vitro, or when the test was performed on unirradiated cells in culture to which the blood serum of irradiated mice was added. These results and the results of three other test methods are compatible with the working hypothesis that low-dose irradiation decreases or inhibits the acceptance of cells for IUdR, and hence also for TdR, and thus increases the pool of TdR in the body fluid. The blood-serum test is simple and fast and is potentially a low-dose short-term, biological accident dosimeter.

Survey on the results in detail:

Whole body irradiation of laboratory animals reduces the whole-body incorporation of I-125-IUdR; the effect depends on radiation dose, radiation quality and time interval between irradiation and injection of the radioactive precursor. The effect is most pronounced for a time interval of 4 hours.

The depression of percursor incorporation is even more drastically demonstrated if an in vivo - in vitro technique is used: 4 hours after whole body irradiation the animals are killed and bone marrow cells are incubated in vitro. This technique is more sensitive and allows the detection of 1 cGy. Irradiation with different radiation qualities were performed and RBE-values were evaluated which increased with decreasing absorbed dose.

Partial body irradiation also leads to a reduced incorporation of the precursor in the shielded body parts.

In 1980 we developed a novel method, which is comparatively simple, very sensitive and unexpensive. The method is based on the discovery that the DNA-synthesis rate of an unirradiated exponentially growing fibroblast culture (L929) depends in a very sensitive way on the addition of blood serum of irradiated mice to a suitable culture medium. Irradiation of the experimental animals leads to a change in the composition of the serum which increases in a still unknown way the inhibition of incorporation of the precursor into the DNA of unirradiated cellls in culture.

Serum from irradiated animals produces a stronger reduction of 125-IUdR incorporation, if added to L929 fibroblast cells in culture, compared to the serum effect from sham irradiated controls. The radiation induced amplification of the serum effect depends on timing, irradiation dose and radiation quality.

Serum from unirradiated mice produces already a strong inhibition of incorporation; e.g. 10% serum results in (71 $\pm$ 2%) depression. After 1 Gy, the effect is even stronger and gives (92 $\pm$ 2%) depression.

Different time intervals between irradiation and serum preparation between 0 and 24 hours were chosen; for a dose of 10 and 100 centigray the maximum effect was measured after

4 hours (Fig. 1). For this time difference of 4 hours between irradiation and blood serum preparation, the inhibition was measured after absorbed doses between 0.006 and 1 Gy relative to the inhibition after addition of unirradiated blood serum (Fig. 2).

The serum experiments do not demonstrate any specific organ reaction, the effect is about proportional to the weight-percentage of the irradiated body.
Using 3H-TdR instead of 125-IUdR leads to an decrease of sensitivity of the serum method. The precursor kinetics in the intracellular nucleotide pool was not influenced by the serum obtained from mice irradiated in the range between 1 and 100 centigray. The radiation induced humoral factor has a molecular weight lower than 15.000 dalton, is not inactivated by heating for 30 minutes at 60° C and produces a competitive inhibition of the incorporation of 3H-TdR.

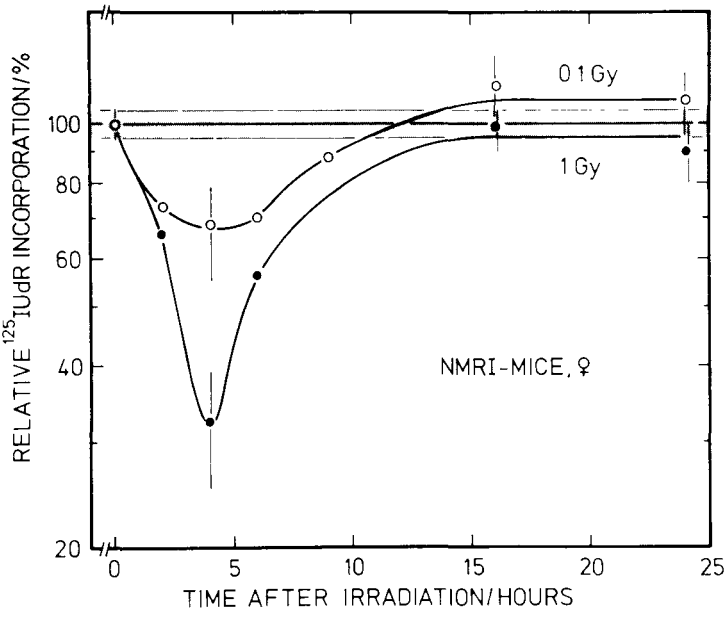

Fig. 1: Time course of the relative incorporation of 125-IUdR into L929 cells in culture.

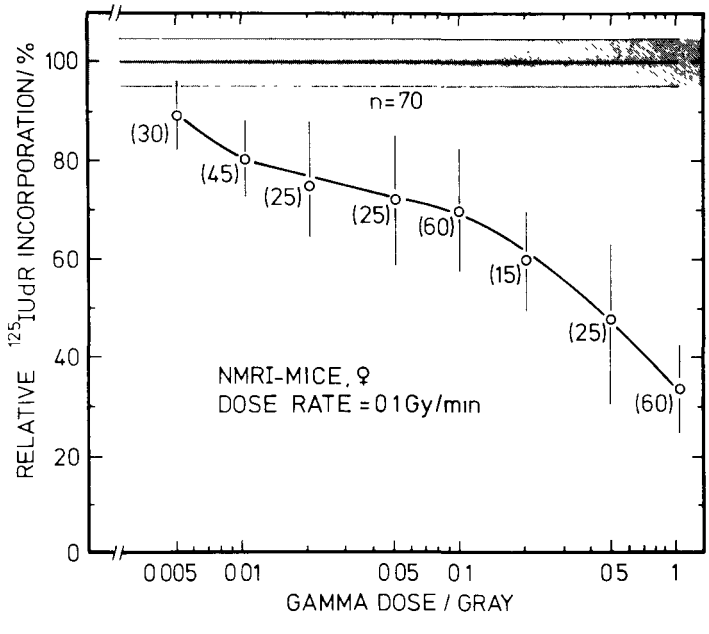

Fig. 2: Relative incorporation of IUdR into L929 fibroblast cells; serum was prepared 4 hours after gamma-irradiation; experimental conditions as in figure 1.

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Contraente della Commissione :

Comitato Nazionale per l'Energia Nucleare, Roma

N. del contratto : 175-76-BIO I
Capo del (dei) gruppo(i) di ricerca : Prof. P. Metalli

Tema general del contratto :

Microdosimetric studies of radiobiological effects at low doses

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Titolo del progetto n.

Microdosimetric studies of radiobiological effects at low doses
Capo progetto e collaboratori scientifici : Prof. M. Coppola
Dr. G. Bertoncello

The contract which has come to an end in December 1980 had its effective beginning during the second half of 1977. By that time, in the framework of the Radiation Protection activities of CNEN a considerable effort had been devoted to the study of biological effects induced by low doses of ionizing radiation, particularly by conventional photon beams and by fission neutrons, as available from the fast reactor facility TAPIRO at the CSN, Casaccia. In addition, high energy neutrons from the Synchrocyclotron of CERN, Geneva, had been and were still employed in biological experiments.

The good quality of the results already obtained, together with the decision to utilize these and other data for a systematic investigation of the influence of radiation quality on the biological effectiveness of ionizing radiation, urged CNEN to include a research project on Microdosimetry among its own research lines. It was, in fact, recognized that for a deep insight in the mechanisms of biological action of radiation, the knowledge of the microscopic patterns in the processes of energy deposition to the biological matter is of a considerable importance. In particular, the measurement of microdosimetric parameters for the radiation fields utilized together with suitably determined biological data, appear to represent a basic information for the construction of models able to predict the level of biological damage produced by radiation with adequate accuracy and, consequently, to allow extrapolation of the results of animal experiments to situations which are experimentally not accessible, and ultimately their use for human risk estimates.

Such a research project, found an excellent location within the lines of activities foreseen in the past Radiation Protection Programme of the European Community, and therefore a contract on microdosimetric studies of biological effects at low doses was stipulated between CNEN and the Commission. The proposed programme offered the additional possibility of canalizing work carried out in different areas towards a well defined common goal. Clearly, the complexity of problems which were to be dealt with was hardly compatible with a short time period such as that still allowed by the past contract, and it was understood that the activities foreseen would be continued either by an extension of the contract itself or as an independent action of CNEN.

In order to limit the area of activity, research was initially concentrated

mostly on effects produced by fast neutrons and reference photon radiation. This choice appeared to be coherent with the recognized necessity for a clarification of some compelling problems existing in Radiation Protection and concerning the assessment of radiation quality for fast neutrons. Therefore, several biological experiments have been planned and carried out under various experimental conditions using monoenergetic neutron beams produced at a Van de Graaff accelerator.

Measurements of biological responses to fast neutron irradiation were performed on various biological systems both "in-vitro" and "in-vivo" using low doses of fast neutrons of various energies. In collaboration with CERN measurements of eye-lens opacification in the mouse were completed and the results were evaluated at 1, 5, and 600 MeV /6/. The experimental observation was based on counting the points of opacities in the posterior region of the murine lenses. It was found that for all neutron energies considered the RBE values tend to increase with decreasing neutron absorbed dose. In contrast with a commonly accepted interpretation, this kind of behaviour could not be explaned by a decrease of effectiveness of the reference radiation at low doses /9/. In addition, at low dose levels, around 1 rad, neutron RBE's for this end-point appear to approach nearly equal values, independently of neutron energy. Conversely, at higher dose levels a marked difference is observed between RBE values corresponding to widely spaced neutron energies as shown in the table.

Within the same collaboration with CERN measurements of dose-response under neutron irradiation were also completed and a data evaluation was carried out for energies ranging from fission up to 600 MeV, for the weight loss of mouse testes 28 days post irradiation /5/. RBE values determined by these measurements were found to depend on neutron energy, with a measured maximum around 1 MeV, however RBE dependence on absorbed dose level was not very significant in the cases considered. The radiosensitive component of the testis tissues shows a very nearly exponential dependence of weight on photon dose over a large interval of absorbed doses. For neutrons, the results seem to suggest an increasing effectiveness per unit dose in the region below about 10 rad.

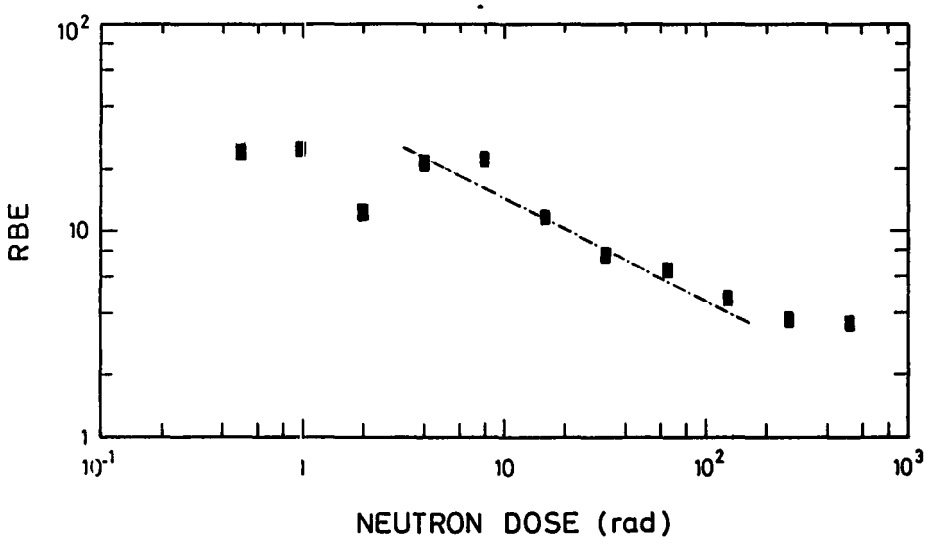
Further studies were initiated meanwhile to investigate neutron RBE for different end points on the mouse. As a complement of the above experiments on eye-lens opacification, additional fast neutron irradiation have been carried out and the biological material prepared to investigate the dose and energy dependence of the number of micronuclei produced in the lens epithelium by fast neutron interactions. Furthermore a large experiemental effort has recently been initiated on the study of induction of ovarian tumors induced by low doses of fast neutrons. Two groups of mice have already been irradiated, one in 1979 and one in 1980, using 1.5 MeV neutrons from a Van de Graaff accelerator, in collaboration with the radiopathology laboratory of CNEN. The results are presently being recorded.

In all experiments already completed the results seem to indicate that neutrons might become increasingly effective when the level of absorbed dose becomes fairly low, at least for some highly radiosensitive biological systems. This observation is in agreement with results obtained by other authors (Shellabarger, C.J., et al., 1980) on rats. Any wider confirmation of such a trend by other evidence could provide interesting matter for rediscussing neutron RBE's at very low dose levels and eventually risk estimates and quality factors. For this reason other measurements have recently been started, in collaboration with the Biology Group at JRC, Ispra, using two diploid species of Nicotiana, whose propoplasts have been irradiated "in-vitro" with 16 MeV neutrons and gamma-rays /11/. In the first experiment the radiation effect was assessed by scoring the relative plating efficiencies. Measurements have been carried out for single acute exposures as well as for fractionated doses. The results already available for N. Plumbaginifolia present no shoulder in the survival curves both for neutron and gamma-rays, and a

Table: neutron RBE for eye-lens opacification

E_n (MeV)	Lower dose region		Higher dose region	
	D_n (rad)	RBE	D_n (rad)	RBE
1	0.9	41.9±7.7	36	18.1±0.6
5	0.9	24.0±6.6	35	11.5±0.2
15	1	33.4±5.2	38	9.5±0.9
400	-	-- --	38	5.9±0.3
600	1	25.7±15.5	41	5.8±0.3

Dose dependence of neutron RBE for N. Plumbaginifolia protoplast survival



markedly steeper slope for neutron doses below about 8 rad. The dose-dependence of RBE from this experiment is shown in the figure. As clearly seen the points are fairly well lying on a segment of $-\frac{1}{2}$ slope for doses between about 4 and 100 rad in the log-log plot. However, also in this case this slope cannot simply be explained by the assumption of a decreasing effectiveness of the reference radiation at low doses. In addition, it is observed that, as expected, fractionation of gamma-ray doses allows a substantial recovery for sufficiently long time intervals between dose fractions. In the case of neutrons, however, the fractionation of absorbed doses in small fractions appears to have no inflence on the neutron effectiveness if the fractions are of 10 rad, but it produces a substantial increase of effectiveness if the fractions are of 5 rad each. These results seem to be consistent with an interpretation suggested by Rossi (Radiat. Environm. Biophysics, 1979) based on the possibility of independence of biological effectiveness of small dose fractions.

Irradiation of Vicia faba roots has also been partially carried out, in cooperation with CERN, in order to compare neutron RBE's in a wide range of neutron beam qualities. Sensitivity of wheat roots has also been tested for growth inibition after 16 MeV neutron and 250 kVp X-ray irradiation. The results have been fully evaluated.

Very recently a study of the biological effectiveness of various qualities of photon radiation has been initiated. New measurements have been performed around the end of 1980 for the weight loss of mouse testes, in a very carefully designed and conducted experiment. Results both for 250 kVp X-rays and Co-60 gamma-rays have already been collected and are presently being evaluated.

Microdosimetric models were applied to the interpretation of cellular inactivation for various cell lines and a large variety of radiation qualities /4/. Saturation of the level of biological effect caused by an excess of energy concentration in the sensitive region of cells was carefully studied and a formulation was suggested which allows a fairly satisfactory prediction of the shape of cellular inactivation curves for high-LET particles. In collaboration with the University of Milan energy deposition spectra in microscopic volumes have been determined for proton beams of various energies and different energy spreads /7/. The results for proton energies up to 31 MeV indicate that distributions are generally skewed with a high-energy tail and that their shapes are markedly dependent on the energy spread of the primary beam.

Finally a steady effort has been devoted to problems related to dosimetry of ionizing radiation, mostly concerning the effects which may influence the response of ionization chambers /8/ /10/. In addition a feasability study for the construction of a high pressure ion chamber has been initiated, in collaboration with TNO.

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Contractor:	Freistaat Bayern, vertreten durch die Julius-Maximilians-Universität Würzburg, Institut für Medizinische Strahlenkunde
Contract No.:	208 - 76 - 7 BIO D
Head of Research Teams:	Prof.Dr.Albrecht M.Kellerer
General Subject of Contract:	Microdosimetric Studies Relevant to Radiation Protection

The two projects in this contract had distinct topics. Project 1 dealt with the radiobiological implications of microdosimetry and with the analysis of radiobiological findings in terms of microdosimetry; it has also largely been devoted to development and application of statistical methods that are required in the derivation of dose-effect and RBE-dose relations for late somatic effects induced by different types of ionizing radiations. Project 2 was aimed at the computation of microdosimetric data and the further development of novel concepts of microdosimetry that permit a more realistic application of microdosimetric data to radiobiology. Certain aspects of the two projects were closely interlinked.

Results of Project No. 1

Head of Project and Scientific Staff: Prof.Dr.A.M.Kellerer,Dr.D.Chmelevsky, Dipl.Phys.T.Fekete, Dipl.Math.U.Mäder, Dr.W.-H.Thomas

Title of Project: Analysis of Late Effects in Terms of Microdosimetry

General remarks:

The main objective of the project has been the analysis of extensive animal experiments aimed at the elucidation of radiation carcinogenesis at low doses of sparsely or densely ionizing radiations. The work has been performed largely in collaborative efforts with the research group of Dr.Shellabarger at Brookhaven National Laboratory and the research group of Dr.LaFuma at CEN-Fontenay-aux-Roses. The results of these studies are described under §§ 1-3. In the last phase of the project a collaborative effort has also been initiated with the research group of Dr.Gössner at GSF; this collaboration is still in its initial phase and no results are listed in this survey, however, it should be noted that certain results on statistical methodology applicable to small samples (see § 7)have been motivated by the collaboration with the group at GSF.

Although the project 1 was aimed primarily at the analysis at late effects and the risks of carcinogenesis at low doses a broad approach was followed and the microdosimetric concepts and mathematical methods were found to be applicable to and relevant to the analysis of primary cellular effects. Problems of particular interest have been treated in collaboration, primarily, with the research team of H.H.Rossi and E.Hall at Columbia University; results of this work are described in § 5.

Problems relating to numerical mathematics and statistics have gained increasing importance in the analysis of radiation carcinogenesis. Essential results in this field are listed under §§ 6 and 7.

§ 1 *Analysis of the induction of mammary neoplasms in the Sprague-Dawley rat by x-rays and by 430 keV neutrons.*

An extensive experiment had been initiated jointly with Dr.Shellabarger from Brookhaven National Laboratory before the beginning of this contract and analysis of the results has been one of the major efforts in project 1. Somewhat less than 1000 Sprague-Dawley rats were exposed to single doses of x-rays between 0.3 and 0.9 Gy or to single neutron doses between 1 mGy and 60 mGy. The choice of the extraordinarily small neutron doses was motivated by the prediction of very high neutron RBE values at low doses and by microdosimetric considerations developed earlier with H.H.Rossi. All animals were followed throughout their life time, up to approximately 1000 days. All mammary neoplasms were registered and classified as benign (fibroadenomas) or malign (adenocarcinomas). Up to 16 independent neoplasms were found in animals exposed to the higher neutron doses. The application of competing risks analysis to these data has substantiated and broadened earlier indications of *sublinear* dose-effect relations for neutrons at small doses. The application of suitable statistical tests for the non-parametric comparison of individual groups has led to the conclusion that the RBE of neutrons at a dose of 1mGy is larger than 100.

The remarkable result has been obtained that the very small single dose of 1 mGy of neutrons accelerates the spontaneous incidence of mammary neoplasms in this rat strain by more than 30 days; a dose of 4 mGy of neutrons produces

a time shift of about 50 days. The sublinearity, i.e. the dose dependence corresponding to higher effects per unit dose at smaller doses, has been established at neutron doses low enough that very few cells receive any energy deposition at all. This proves that the radiation induced neoplasms are due not merely to cellular transformations but that tissue effects must govern the dose dependence.

The high RBE values for neutrons exceed the conventional quality factors in radiation protection substantially, and are of obvious importance to risk estimation. The sublinearity of the dose-effect relation for neutrons may have an even greater potential impact on the entire philosophy of linear estimates in radiation protection.

§ 2 *Induction of mammary neoplasms in the ACI rat by neutrons and x-rays and the problem of synergism with DES*.

The extraordinary nature of the results described under § 1 has motivated an additional effort aimed at a comparative investigation in a different animal strain with almost no spontaneous incidence of mammary neoplasm but with a substantial induction of mammary neoplasms by the synthetical hormone DES. This hormone has found particular attention because of its wide spread, although largely illegal ,use in animals and it is also considered within the framework of possible synergistic effects relevant to risk assessment for routine mammography.

The experiment has been analogous to the experiment described under § 1 and was also conducted in Brookhaven and analysed in Würzburg. ACI rats have been exposed to neutrons or x-rays and part of the animals were also treated by DES. The low incidence in the non-DES treated animals necessitated neutron doses in excess of 30 mGy and in this dose range neutron RBE values close to 10 were obtained; this is in agreement with the results for Sprague-Dawley rats. For the DES treated animals RBE values and the shape of the dose effect relation have been obtained at small neutron doses down to 10 mGy and were substantially in excess of 100. This is higher than the values obtained for the Sprague-Dawley rats at the same neutron dose. Furthermore a substantial sublinearity of the dose-effect relation for the neutrons has again been

established. As in the Sprague-Dawley rats, the results for x-rays are consistent with an approximately linear dependence on x-ray dose.

The observation of even higher RBE values and the reconfirmation of a sub-linear dose effect relation for neutrons in a different system are important, and demonstrate that the results described under § 1 are not restricted to one particular strain. In addition the results point to the necessity of a more detailed analysis of RBE values for densely ionizing radiation in synergistic effects.

The essential findings are about to be published; but a further combined analysis of this experiment and of the earlier Sprague-Dawley results will have to be performed with main emphasis on the comparative dependence of tumour rates on age of the animals after irradiation.

§ 3 *Analysis of pulmonary neoplasms .*

A considerable effort within the research project has gone into the analysis of large inhalation experiments earlier performed by Dr.LaFuma et al. at CEN-Fontenay-aux-Roses. The assessment of pulmonary neoplasms in rats is made particularly difficult by the fact that these tumours are not correlated with mortality and are found only incidentally in sacrificed animals or in animals dying for unrelated causes. This condition corresponds to the problem that, in statistical theory, is termed the analysis of *double censored* data. Tests and estimation procedures for this type of data are insufficiently developed and work along these lines has been a major part of the project (see §§ 6 and 7). An analysis has first been applied to a particularly important part of the extensive data sets, namely to the radon inhalation studies. The results are now being submitted for publication. The essential achievement has been the estimation of the prevalence for radon induced malign pulmonary neoplasms as function of dose and of time after radon inhalation.

Three models have been tested. The best fit has been obtained with the assumption of a dose dependent acceleration factor for the tumour prevalence. However, a dose dependent time shift, and the more commonly considered

proportional-hazards model can also be utilized, and it is remarkable that mortality corrected integral incidences computed on the basis of the different models are in substantial agreement. A risk factor for malign pulmonary neoplasms in the Sprague-Dawley rat of $2 \cdot 10^{-4}$ per WLM (working level months) of radon inhalation is obtained. It is particularly noteworthy that even at smallest doses where life shortening plays no role the competing risk analysis leads to improved results. The reason is the inherent stratifying effect that corrects for random fluctuations of life time within experimental groups.

It is felt that the methods which have been exemplified in the application to the radon results will be equally applicable to other inhalation studies or to the assessment of pulmonary neoplasms induced by external irradiations. This is important in view of the fact, that extensive experimental data have, for a considerable time, remained unutilized, except for a simplified analysis in terms of life shortening that is essentially unrelated to the incidence of pulmonary neoplasms.

§ 4 *Analysis of leukemia and other neoplasms in the survivors of the atomic bomb explosions.*

Extensive methodological work has been done on the evaluation of these data. It has been found that most of the earlier analyses, although highly valuable, are in need of a reassessment, particularly with regard to the determination of confidence intervals for the dose-effect relations and the RBE-dose relation. In view of ungoing far-reaching reevaluations of dosimetry (particular the 'Livermore study') it was felt that actual numerical evaluations should await the outcome of these dosimetric reassessments. The numerical analysis will be the objective of a separate effort in the new research period that will be performed as a collaborative task with RERF and with Columbia University.

§ 5 *Further development of microdosimetric concepts and application to radiobiology.*

The notion of the proximity function has been utilized for a more general formulation of the dual-action theory. This treatment is applicable to experiments with short ranged electrons released by ultra-soft x-rays.

However, an important application, conducted jointly with Columbia University, has dealt with the results of experiments where cells were subjected to pairs of correlated deuterons in order to determine the effect as a function of the mean separation of the ions. The detailed analysis has been published for the cell survival studies; the results for the cytogenetic studies are similar. The principal result of the analysis is that the effect is largely due to interaction of damage in the cell over short distances up to 0.1 µm. However, it is also shown that independent charged particles interact over much larger distances to cause the quadratic component of the dose-effect relation. The result is of particular pragmatic interest as it demonstrates that the two types of damage, being of different nature, may be subject to different dose modifying factors.

The analysis of the molecular ion beam experiments has been largely based on non-linear optimization procedures. The same algorithms play an important part in the carcinogenesis studies; they have also proved valuable for a non-parametric representation of dose-effect relations, in general. A determination of dose-modifying factors that necessitates no assumption of analytical expressions has been applied to numerous experimental investigations in a joint investigation with E.Hall, Columbia University. The method can remove the often critical problem of the somewhat arbitrary choice of equations in the study of dose-effect relations.

§ 6 *Statistical estimation procedures.*

Non-linear optimization methods based on maximum likelihood and on the method of steepest descent or on related algorithms have been applied widely in the project for the analysis of radiation carcinogenesis. The methods have proved essential in estimating the age dependence and the dose dependence of integral tumour rates and of tumour prevalences. They are particularly suitable in the comparison of models, such as the proportional hazards model and those of time shift or acceleration of tumour rates or prevalences (see §§ 1-3). Numerical convergence of the algorithms can be problematical in the analysis of large data sets. Considerable effort has therefore gone into the refinement and the thorough testing of the algorithms. They are, by now, a highly important and broadly applicable tool at this laboratory.

§ 7 *Non-parametric tests for the comparison of tumour rates.*

The Mantel-Haenszel test or the equivalent log-rank test and the similar tests by Gehan and Breslow are of increasing importance in the analysis of radiation carcinogenesis. We have attempted to find corresponding tests applicable to double censored data. Certain empirical procedures were found that appear to work satisfactorily in a variety of cases. Nevertheless we have failed to find a test of general validity. The problem deserves further study although the existence of a solution is not certain.

We have, however, made substantial progress in developing small sample tests applicable to right censored data that are analogous to the log-rank or the Breslow test. These tests are valuable for radiation carcinogenesis studies with relatively small numbers of animals. The method underlying the small sample tests is the computation of exact distributions by successive convolutions. The power functions of the small sample tests have been investigated for a number of representative cases. The bias of the results due to the interdependence of sample sizes in an ungoing experiment has been investigated, and it has been found to cause no difficulty in the small sample version of the Mantel-Haenszel or log-rank test. For the small sample test analogous to the Breslow test the bias can be serious when samples of very different size are compared; however, the problem disappears in the usual case of strong censoring.

The results are being prepared for publication, and it is felt that they will be of importance for those increasingly required carcinogenesis studies where multiple factors are tested and multiple small groups of animals must therefore be compared.

Results of Project No. 2

Head of Project and Scientific Staff: Prof.Dr.A.M.Kellerer,Dr.D.Chmelevsky, Dipl.Phys.T.Fekete,Dipl.Math.U.Mäder, Dipl.Phys.H.Roos, Dr.R.Silbermann, Dr.W.-H.Thomas, Dr.F.Zilker

Title of Project: Computation of Microdosimetric Distributions for Tissue Regions of Diameter 1 to 100 nm

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General remarks:

The purpose of this project was more narrowly defined than the broader objective of project 1. However, the project was not limited to the mere computation of microdosimetric data; it was equally dependent on a general effort to widen the framework of concepts and quantities utilized in microdosimetry and applicable to radiobiology (see §§ 1-3). As an unforeseen additional result, close connections between microdosimetry and conventional dosimetry have become apparent; this is described under § 4. It is felt that the newly recognized interrelations will be fruitful both for dosimetry and microdosimetry. § 5 indicates work performed under the project that belongs to the field of geometrical probability and that is relevant both to dose calculations and to microdosimetry computations. There are also substantial interrelations with problems arising in automatic pattern recognition and in texture analysis.

§ 1 *Further development of the concept of proximity functions.*

The novel concept of the proximity function, i.e. the distance distribution between pairs of energy transfers in charged particle tracks, has opened various new possibilities in microdosimetry and its application to radiobiology. An application of particular importance to radiobiology generalizes the earlier microdosimetric treatment of the interaction of sublesions in the cell; it has been discussed in § 5 of the report to project 1. The same formalism is applicable to the analysis of other radiation effects, for example those on solutions of DNA. In view of such applications the problem of diffusion of imparted energy or radiation induced free radicals has been studied, and a mathematical formalism has been developed that expresses the resulting transformation of the proximity functions. The formalism has been utilized for a comparison of computed functions with proximity functions observed in cloud chambers; it has also been utilized to evaluate the radiobiological implications of energy migration on the nano-meter level.

It has been shown that the proximity functions permit the computation of dose-average energy imparted or lineal energy in arbitrary volumes; and computation of this type have been performed.

A particularly straight forward and important application of the proximity functions is the assessment of the degree of equivalence between microdosimetric spectra obtained with spherical and cylindrical proportional counters. It has been concluded that the more complicated and expensive spherical counters can be largely replaced by cylindrical counters. The dose averaged mean values of the microdosimetric spectra differ by less than 2 % for any type of radiations when a detector sphere of equivalent diameter d is replaced by a unit elongation cylinder of height and diameter 0.87 d. The result has obvious importance for experimental microdosimetry.

§ 2 *Computation of proximity functions for electrons.*

A systematic evaluation of proximity functions for electrons between 100 eV and 10 keV has been performed in a joint project with Drs.Terrissol and Patau, Toulouse University. These calculations include also the determination of basic characteristics of the simulated tracks. Furthermore the more conventional microdosimetric parameters are computed, and comparisons of the results have been performed with data obtained earlier. The work is considered as a first step towards the systematic tabulation of proximity functions for various radiations.

§ 3 *Proximity functions for neutrons and related distributions.*

Proximity functions for neutrons have been computed in the same LET approximation that has been utilized in computations of microdosimetric spectra by Caswell et al. It has been shown that, for neutrons, the concept of the proximity function is subject to restrictions due to saturation effects in the biological action of densely ionizing particles. It has therefore been found necessary to deal with a more explicit analysis in terms of distributions of energy around energy transfers chosen at random within particle tracks.

These functions, that can be termed centered distributions, have been computed for neutrons and have been compared to the conventional microdosimetric distributions.

The results for neutrons will have to be extended by computations that include also the fine structure of the tracks. This factor is essential for data relating to small distances in the nano-meter region.

§ 4 *Mathematical analogies between microdosimetric computations and dose computations with internal emitters.*

Microdosimetric formulae invoke the proximity functions for radiations and the analogous proximity functions for receptor volumes. These equations have striking similarities to formulae that are important in dose calculations with internal emitters. In the dose calculations the concept of the point-pair distributions of geometrical objects and the geometric reduction factor have earlier been introduced by M.J.Berger and they have attained considerable practical importance. It has now been shown in work on this project that there are substantial mathematical analogies between the mathematical relations in microdosimetry and in dosimetry. The geometric reduction factor can be expressed in terms of the proximity function of receptor structures. The computations of the proximity functions or the geometrical reduction factors are therefore equally relevant to microdosimetry and to dose calculations with internal emitters. The problems of geometrical probability arising in this context are also closely linked to a field of mathematics that has been termed mathematical morphology. These matters have in essential points been published, but will require further study.

§ 5 *Proximity functions for general cylinders.*

Proximity functions and the related geometric reduction factors, have been computed for cylinders. The solution has similarities to earlier work that led to a solution for the so-called chord length distribution for cylinders. However, the resulting formulae are substantially simpler than the earlier solution for the chord-length distributions. A systematic investigation of

the connection between receptor-proximity functions and chord-length distributions for different types of randomness has been performed and published together with the results for general cylinders.

The results are of practical importance for dose-calculations and for microdosimetric computations for cylindrical detector volumes. As stated in § 1, they are also utilized in the assessment of equivalence between microdosimetric spectra obtained with cylindrical and spherical detectors.

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Vertragspartner der Kommission:	Physikalisch-Technische Bundesanstalt, Braunschweig
	Gruppe für Photonen- und Elektronendosimetrie
Nr. des Vertrags:	209-10-76 BIO D
Leiter der Forschungsgruppe:	Prof. Dr. Herbert Reich
Allgemeines Thema des Vertrags:	Dose distributions at interface
Titel des Projektes Nr. 1:	Dosisverlauf an Grenzschichten aus Ionisationsmessungen
Leiter des Projektes:	Prof. Dr. Herbert Reich
Wissenschaftliche Mitarbeiter:	Dr. Jürgen Böhm Dr. Klaus Hohlfeld Dr. Manfred Schneider

This project was undertaken to determine the ion dose distribution in the vicinity of an interface between two media of different atomic number irradiated by a photon or electron beam travelling normal to the interface. The local variation of dose is limited to the region of electron non-equilibrium which exists on either side of the interface. The thickness of the transition region is of the order of the maximum range of the secondary electrons.

In radiological protection for evaluating the effective dose equivalent, the mean doses in various tissues or organs are considered by using weighting factors. In evaluating the mean doses in various tissues of the body the interface effects are not taken into account. They may be serious when the atomic numbers are quite different, as in the case of bone and cells on bone surfaces. For the blood forming cells in the red bone marrow, a weighting factor four times higher than for the bone itself has to be applied (e.g. ICRU Publ. 26, 1977).

Measurements of the interface effect are rather delicate. Ionization in gaseous layers was used to expand the geometrical dimensions. A precision double extrapolation chamber, consisting of two component chambers, allowed the gas pressure and the depths of the two component chambers to be varied /2/. By the compensation of the main portions of the currents in both component chambers, the double chamber made it possible to measure the changes of the currents in one component chamber only. Thus the ionization in thin layers was accurately detectable, filling the gap between earlier measurements of the current in vacuum chambers and ionization currents in gas filled chambers. Measurements at distances between 10 nm and 100 µm from the interface have been lacking until now.

At low gas pressure, measurements of the ionization currents are disturbed by other currents. A method was developed to evaluate the influences of the low-energy non-ionizing secondary electrons liberated from the walls. It is based on a model of charge transport within a gas which takes into account the effect of back diffusion (the motion of charge carriers against the forces of the electric field towards the electrode of their origin). With this method it was possible to determine the ion dose distribution in the gas close to a tin-air interface down to a distance of about 10 nm referred to an air density of 1 g/cm^3. The method is suited to the investigation of interface effects for all kinds of radiation (gamma and X rays, electrons and perhaps neutrons).

Der Dosisverlauf im Gas in der Nähe von Festkörper-Gas-Grenzschichten konnte mit Hilfe von Extrapolationskammern bisher nur für Abstände von der Grenzschicht bis herab zu etwa 100 µm gemessen werden. Darunter ließen die Unsicherheit der Ortsbestimmung sowie störende Elektronenemissionen aus den Wänden keine Aussagen mehr über den Dosisverlauf zu. Durch Einführung eines Kompensationsverfahrens für die Kammerströme und Übergang zu niedrigen Gasdrücken konnte der Untersuchungsbereich bis herab zu etwa 10 nm Abstand von der Grenzschicht (bezogen auf die Gasdichte 1 g/cm^3) erschlossen werden.

Im Gegensatz zu üblichen Extrapolationskammern mit zwei planparallelen Elektroden wurden hier drei Elektroden so zusammengefügt, daß zwei planparallele Kammern (Doppelkammer) mit gemeinsamer Mittelelektrode entstanden; die Mittelelektrode dient als Meßelektrode. Durch geeignete Wahl der Elektrodenmaterialien tritt ein Übergangseffekt in der Nähe der Grenzschicht nur in der zuerst durchstrahlten Teilkammer auf, deren Vorderwand aus dem zu untersuchenden Material besteht. Die Mittelelektrode und die Hinterwand der zweiten Kammer bestehen aus einem gasäquivalenten Material. Die durch den Grenzschicht-Effekt nicht beeinflußten Ionisationsströme in den Teilkammern können durch Differenzschaltung kompensiert werden.

Zur Untersuchung der Dosis in der grenzschichtnahen Zone werden zwei Parameter geändert: das Kammervolumen durch Änderung des Elektrodenabstandes und die Gasdichte durch Änderung des Drucks. Dazu wurde die Apparatur in einem evakuierbaren Gefäß aufgebaut, in dem sich der Druck zwischen 0,001 mbar und 1 bar einstellen ließ. Bei geringem Plattenabstand, kleinem Gasdruck und in Kompensationsschaltung treten sehr kleine Ströme auf, die genau zu messen sind. Zur Kalibrierung des Strommeßsystems mußte eine Referenzstromquelle entwickelt werden /1/. Der Meßaufbau mit der Doppelkammer ist in der Fig. 1 dargestellt, eine ausführliche Beschreibung anderweitig veröffentlicht /2/. Bei vermindertem Druck treten in Ionisationskammern Störeffekte durch aus den Elektroden stammende Sekundärelektronen auf. Die Energie der Elektronen reicht nicht aus, um das Gas zu ionisieren. Unter Vakuumbedingungen läßt sich dieser Elektronenstrom zwar messen, jedoch

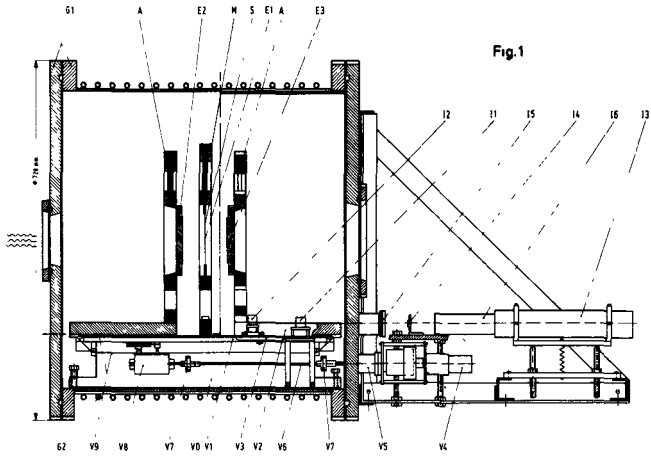


Fig. 1: Schnitt durch die Apparatur.
G = Gefäß zur Aufnahme der Doppelkammer, G1 = Vakuumgefäß , G2 = Heizspirale zur Temperaturstabilisierung und Ausheizung.

E = Elektroden der Ionisationskammer:
E1 = Kohlenstoff-Meßelektrode, umgeben vom Schutzring S, E2 = Zinn-Eintrittsfenster, E3 = Kohlenstoff-Austrittsfenster, M = Mittelelektrodenhalterung, A = Außenelektrodenhalterung, verschiebbar.

Verschiebevorrichtung V für Kammertiefen.
V0 = Verschiebebank mit Führungsschiene V1 und zwei Verschiebewagen V2, die auf Kugelführungen V3 laufen. Sie werden über Motor und Getriebe V4, Drehdurchführung V5, Achse V6, Kardankupplung V7, Schneckengetriebe V8 und Zugseil V9 bewegt.

Interferometrische Messung I der Kammertiefen:
Zwei Laser-Interferometer, jeweils bestehend aus Strahlteiler I1 und Reflexionsprisma I2. Als Lichtquelle dient ein He-Ne-Laser I3, dessen in I6 aufgeweiteter Strahl durch ein Glasfenster I5 auf den Abtastdioden I4 Interferenzsignale erzeugt, die die Verschiebung und ihre Richtung angeben.

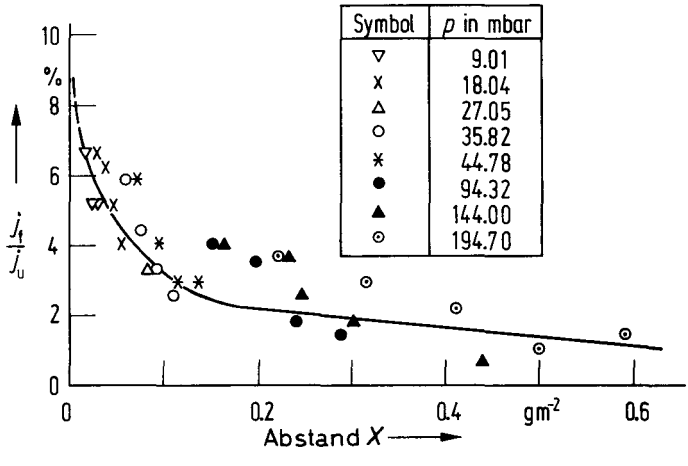


Fig. 2: Ionendosis J_f in Prozent der ungestörten Ionendosis J_u an der Zinn-Luft-Grenzschicht in Abhängigkeit vom Abstand von der Zinn-Elektrode (x = 0). Die Symbole entsprechen unterschiedlichen Versuchsbedingungen bezüglich Druck und geometrischer Kammertiefe.

wird bei vorhandener Gasfüllung ein Teil davon durch Anlagerung an diffundierende Gasmoleküle gegen die Krafte des elektrischen Feldes zu der Elektrode, aus der er hervorgegangen ist, zurückbefordert (back diffusion). Dieser Anteil ist druck- und feldstarkeabhängig und wurde ausführlich in einer anderen Arbeit /3/ untersucht. Bei der aus drei planparallelen Elektroden bestehenden Kammer laßt sich bei geeigneter Wahl der Polaritaten der beiden Teilkammerspannungen eine Einstellung der Kammertiefen finden, in der sich die Störeffekte nahezu aufheben (compensation mode).

Im "compensation mode" wurde für ^{90}Sr + ^{90}Y-Betastrahlung an einer Zinn-Luft-Grenzschicht der Ionisationsstrom in Abhangigkeit von der flachenbezogenen Masse der Luft gemessen. Die in Strahlrichtung erste Teilkammer, gebildet durch das Zinn-Eintrittsfenster und die Kohlenstoff-Mittelelektrode, wird im folgenden als Sn-C-Kammer bezeichnet, während die zweite durch die Mittelelektrode und das Kohlenstoff-Austrittsfenster gebildete Kammer als C-C-Kammer bezeichnet wird. Die Ionendosis an der Grenzschicht kann als die Summe J_t (total) der ungestörten, in der Kammer konstanten Ionendosis J_u und dem durch die Grenzschicht zusatzlich entstehenden Anteil J_f (Folie) aufgefaßt werden. Durch Variation der Kammertiefe x_c der C-C-Kammer laßt sich bei konstanter Kammertiefe x_t der Sn-C-Kammer und bei konstantem Druck p die ungestörte Ionendosis J_u bestimmen. Bei demselben Druck und fester Kammertiefe x_c läßt sich dann die auf J_u bezogene Ionendosis J_t der Sn-C-Kammer als Funktion der Kammertiefe x_t und damit direkt die Ionendosisverteilung J_f an der Zinn-Luft-Grenzschicht für ^{90}Sr + ^{90}Y-Betastrahlung ermitteln (Fig. 2). Vor der Auswertung wurde die Divergenz des ß-Strahlenbundels, die fur gleiche Kammertiefen unterschiedliche Strome in beiden Teilkammern bewirkt, durch eine Korrektion berücksichtigt.

Da J_u mit dem Extrapolationsverfahren sehr genau bestimmt werden kann, rührt der Hauptanteil der Meßunsicherheit fur J_f aus der Bestimmung von J_t her. Eine weitere Methode besteht in der Messung des Ionenstromes bei Variation des Drucks des Fullgases bei nahezu gleichen Kammertiefen beider Kammern. Sie liefert das Integral der Ionendosis über die flächenbezogene Masse. Dieses Verfahren zeichnet sich durch kleine Unsicherheiten der Meßwerte aus, besonders bei niedrigen Gasdrücken, bei denen die Ströme bei

wenig mehr als 10^{-15} A liegen. Die Verfahren zur Bestimmung der Ionendosisverteilung aus den gemessenen Ionisationsströmen und die sich daraus ergebenden Resultate für eine Sn-C-Grenzschicht sind in /4/ veröffentlicht. Das Ergebnis zeigt, daß die Ionisationsmethode geeignet ist, die Energieabsorption in Volumina mit linearen Abmessungen von ca. 20 nm bei einer Dichte von 1 g/cm^3 zu untersuchen. Die Umrechnung der Ionendosis in die eigentlich gewünschte Energiedosis ist z.Z. nicht möglich, da für das hier vorliegende Spektrum von Elektronen niederer Energie bisher keine Werte für die mittlere Energie zur Erzeugung eines Ionenpaares bekannt sind.

Auch nach dem zeitlichen Auslaufen des Vertrages werden die Arbeiten weitergeführt und Grenzschichteffekte für andere Strahlenarten und andere Materialkombinationen untersucht.

Veröffentlichungen aus diesem Vertrag

/1/ Böhm, J.
Eine Referenzstromquelle zur Kalibrierung von Strommeßsystemen in der Dosimetrie mit Ionisationskammern, PTB-Mitt. 89 (1979), S. 237-241 und PTB-Dos 2, Febr.1979 (ausführliche Darstellung).

/2/ Schneider, M.; Böhm, J.; Hohlfeld, K.; Reich, H., S. 355 in den Proceeding of the Sixth Symposium on Microdosimetry Brussels, Belgium, May 22-26, 1978. Ed. J. Booz, H.G. Ebert. EUR 6064 DE-EN-FR

/3/ Bohm, J.; Schneider, M.; Hohlfeld, K.; Reich, H.
Ionization current measurements at low gas pressures and small chamber dimensions. Proceedings of the Seventh Symposium on Microdosimetry, Oxford, September 8-12, 1980. Ed. J. Booz, H.G. Ebert (im Druck).

/4/ Schneider, M.; Böhm, J.; Hohlfeld, K.; Reich, H.
Experimental determination of the ion dose in the vicinity of a tin-air-interface. Siehe /3/.

Contractor: Department of Medical Biophysics, The University, Dundee, DD1 4HN. U.K.

Contract No: 184-76-7 BIO UK

Head of the research team(s): Dr. D.E.Watt.

General subject of contracts: Fundamental dosimetric studies with slow heavy charged particles.

Title of the project nr.1: Condensed phase effects on stopping cross-sections for heavy charged particles.

Head of project and scientific staff: D.E.Watt, D.I.Thwaites, T.K.Yeung and H. Iskef.

Introduction.

Stopping power is a basic physical parameter required for computation of the structure of charged particle tracks and of various dosimetric quantities such as dose, slowing down spectra, and quantities which may represent quality e.g. LET and microdose quantities. Stopping power ratios (wall medium to gas filling), required for cavity chamber dosimetry, could be influenced by the physical phase and chemical structure of an absorber.

The objectives of the programme were

(i) to provide stopping cross-section data for light ions at energies near and below the Bragg peak in materials of dosimetric interest,

(ii) to explore possible differences in the stopping power of a medium attributable to (a) its physical state (phase effect) and/or (b) the chemical bonding (i.e. resulting in deviations from Bragg additivity). Phase and binding effects are expected to be only a few percent and so highly accurate measurements of stopping power are mandatory.

Approach and Results.

A detailed correlation of all relevant stopping power information published prior to 1977 was carried out in a rather powerful way, using Lindhard reduced functions, to evaluate existing evidence for a physical phase effect and to resolve the conflicting experimental conclusions.[2,4] Positive evidence was obtained which showed that the stopping powers in condensed /

condensed media of low atomic number, for H, He and N ions respectively were a maximum (near tht Bragg peak) of 10%, 15% and 36% less than in the equivalent vapour. The analysis showed also that the spread of published data for gases was significantly greater than for solids (thin films) demonstrating unequivocally that there were unsuspected systemic errors and indicating the need for more accurate stopping power measurements.

An effective stopping power ratio (vapour to condensed phase) derived for neutrons in tissue-like media decreased from a maximum of 1.08 at 0.1 MeV to a constant value of 1.03 at energies above 5 MeV.[1] The magnitude of the phase effect increased with projectile atomic number and is greatest in light media. Anomalous results observed in solid media are attributed to differences in the degree of atomic order within the structure of thin films compared with the bulk medium.

Bethe theory is found to remain applicable to the condensed phase for fast particles if the mean excitation potential I is modified on the assumption that inner shell electrons are unaffected by aggregation and that a collective excitation energy (e.g. plasmon energy or the maximum in the oscillator strength distribution) can be substituted for the contribution from the outer electrons.[3]

Two experimental rigs were constructed for measurements of stopping power in media of gaseous, liquid and solid phases. The first was an indirect method (operational in 1977) in which the measured residual particle energy for increasing absorber thickness was fitted by an analytical expression and differentiated to yield stopping power. Data can be obtained for vapour and liquids in the same apparatus. The other method for vapours and solid films yields a direct measure of energy loss by particles which are energy selected in a high resolution magnetic analyser (operational in 1980).[5] Main problems specifically identified and solved were inaccuracies of previous work due to inadequate curve-fitting and uncertainty in the role played by multiple elastic scattering at low projectile energies.

Multiple elastic scattering.

Multiple elastic scattering becomes of increasing importance with decreasing /

decreasing particle energy. Detailed information is required in dosimetry for track structure calculations; to obtain correction factors to energy loss and transmission measurements in thin films for stopping power determinations; for separation of electronic and nuclear components of stopping power. As no previously published information was available in the energy region of greatest interest ($\alpha = Z_1Z_2/137\beta > 1$) a new model was developed to find the multiple scattering corrections (δE_{ms}) to the energy loss experienced by heavy charged particles penetrating through thin films. This model incorporated the Sigmund-Winterbon theory of multiple elastic scattering,[11,13] which on detailed analysis, was considered to be the most appropriate.

δE_{ms}% was evaluated for various projectile - target - energy combinations, namely H, He, Li, C, N and O ions at energies in the range 0.01 to 2 MeV in H, O, Al, Ar, Ni, Ag and Au, for circular detectors with angles of acceptance (ν) in the range $0.05 \leq \nu \leq 0.3$ and for energy losses from 20% to 80% of the incident particle energy. The corrections can amount to a few percent when energy loss in the absorber, and/or the detector acceptance angles, are large.[13,14,15] This work has been successfully extended to cope with compounds. Results for water are not much different from, but somewhat smaller than, those for oxygen. Calculation of δE_{ms} for some hydrocarbon compounds is near completion.

Numerical data is obtained also for the fraction of particles (N_m%) scattered out of the detector acceptance angle. This parameter could have important consequences in transmission measurements and in determination of the spatial distribution of radiation damage. N_m has been determined for protons and alpha particles of various incident energies, detector acceptance half-angles and target media. In general N_m increases with target atomic number for the same particle energy.[14]

Cross-section and range measurements.

Proving experiments were performed by measuring stopping cross-sections for alpha particles (0.2 to 5.5 MeV) in He, N_2, Ar, CH_4 and Air using the indirect method. Careful overall assessment of measurement /

measurement and data analysis procedures was made including comparison with other published results and indicated that the absolute uncertainty of the techniques employed could be as much as 5% over and around the Bragg peak. Ranges are accurate to about 2%. This work provided additional basic data and established a rigorous procedure for accurate use of the indirect method.(7)

New cross-sections, obtained for the energy loss of alpha particles (0.3 to 5.5 MeV) in liquid water and vapour, demonstrated a clear phase effect of up to 4% increase in stopping for 1.75 MeV alpha particles in water vapour. The trend continues to increase towards lower energies.(6)

All the major problems of accurate measurement are believed to have been overcome and an on-going programme of data accumulation is now in progress using both the direct and indirect methods. Measurements are being made in a number of hydrocarbons in vapour, liquid and solid forms. These include single, double and triple bonded straight chain compounds. Methods have been developed for the production of thin films of accurately known uniformity and thickness.

During 1980 a study began on the stopping power of electrons at energies below 10 keV. Theoretical treatments are self-consistent above 200 eV but the published experimental data has serious shortcomings being both inconsistent and insufficiently comprehensive particularly for organic materials.(10) An electron accelerator has been constructed to provide 20 μA beams of electrons variable in energy between 100 eV and 10 keV with an energy resolution of about 1%. Energy loss is measured using calorimetry techniques. Preliminary measurements now in progress should ultimately yield valuable information pertaining to δ-rays.

The programme as a whole has demonstrated successfully the existence of phase effects and established the magnitude of these in some important instances. New basic cross-section data has been obtained for alpha particles. Extension of the work to other media and particle types is likely to continue for several more years.

Title of project nr. 2: Ionization cross-sections and W values in gases for heavy ions (< 100 keV).

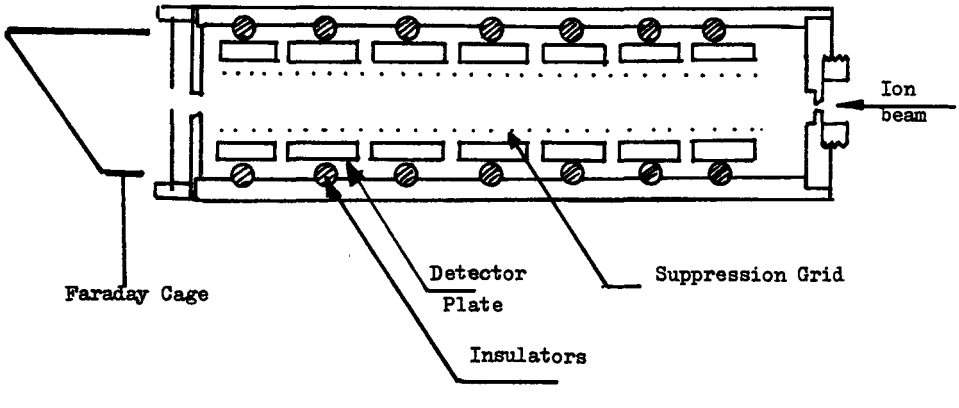
Head of project and scientific staff: S. Hughes, B.C.Edmans and D.E.Watt

This project is aimed at obtaining experimental and theoretical integral W(E) and differential w(E) values for the mean energy expenditure per ion pair for light ions with energies in the range 5 - 100 keV in gases of dosimetric interest for application to neutron dosimetry. Additionally the work was intended to relate basic physical interactions to the production of molecular fragments in gaseous molecules for guidance in the development of track structure theory and in our understanding of radiation damage mechanisms.

The relative abundance of positive ion collision products was measured for H^+ and N^+ in CH_4 and C_2H_6. From the trend of the individual fragment cross-sections it was concluded that charge exchange, both simple and dissociative, was a major process within the ion energy range under single collision conditions. Of the ionization processes possible a large proportion of interactions result in dissociation of the target molecule. Production of free H^+ ions was unexpectedly large ($\sigma \sim 2 \times 10^{-16}$ cm^2).

For the measurement of W values, a parallel plate detector with secondary electron suppression grids was designed, constructed and given initial tests. Details of the construction are outlined in Fig. 2.1.

Fig.2.1



This detector is intended for measurement of differential W values along track segments as well as total W values by collection of total positive and negative signals generated by collimated beams of H^+, N^+ ion in organic gases and tissue equivalent gases. The presence of the two grids enable suppression of secondary emission from the collector plates.

Test measurements, however, indicated faults in the assembly which resulted in unacceptably high background signals. This detector has since deen re constructed with suitable modifications and is now ready for use but has not been tested in its modified form. A further period of time will be required for completion of the intended measurements.

In parallel to the above a thorough review has been completed of the wide range of theories having possible relevance to producing consistent calculated values of W.

The objectives of this part of the workwere to review the existing theoretical models for explicit calculations of W values (e.g. Fowler, Kmipp, Gerhart, Garvey & Green) and to investigate other methods which may be more useful for the energy region and ion-target consideration of interest to this work.

The approach adopted has been to calculate

(i) relevant ionisation cross sections, and from these

(ii) the number of ion pairs produced, initially taking account of primaries only, with subsequent estimates of secondary and tertiary ionisation.

From these are calculated

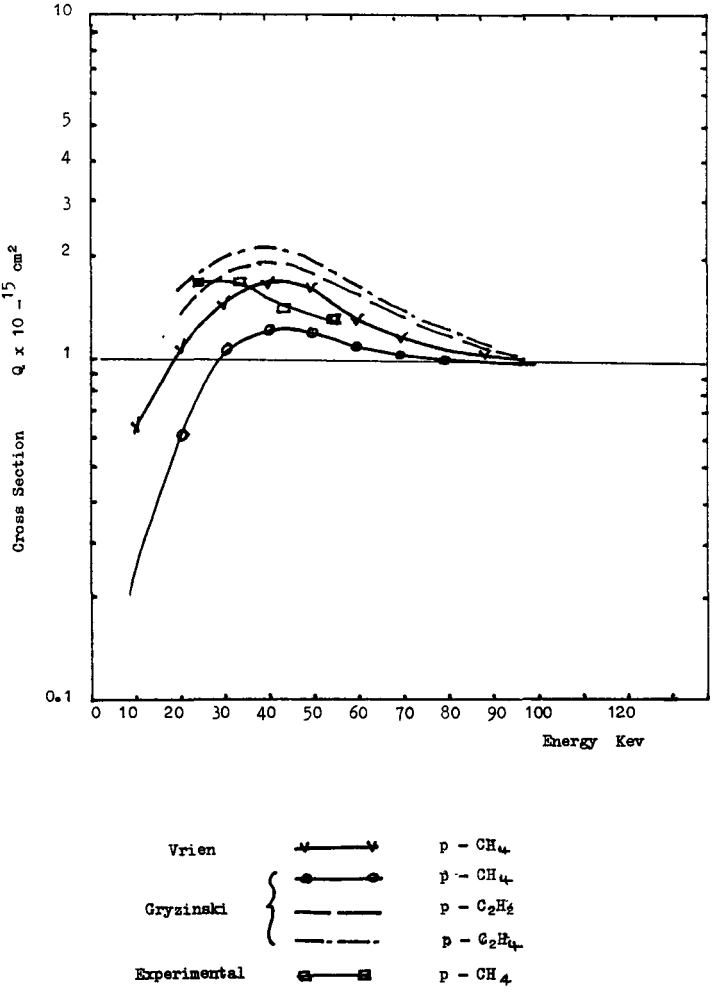
(iii) stopping powers, estimates of

(iv) W values are produced.

Comparison of results with experimental data where available will indicate the usefulness of the methods employed.

So far results have been obtained for ionisation cross sections for protons in CH_4 using modified Gryzinski and Vriens and by scaling these for protons in C_2H_2, C_2H_4, . Fig.2.2

Fig. 2.2


10
5
4
3
2
1
0.1
Cross Section Q x 10 $^{-15}$ cm^2
0 10 20 30 40 50 60 70 80 90 100 120
Energy Kev
Vrien
p - CH_4
Gryzinski
p - CH_4
p - C_2H_2
p - C_2H_4
Experimental
p - CH_4

Approximate calculations for He, C, N, O have also been obtained for CH_4 target gas, but are not considered to be reliable, due to the probable effects of projectile ion structure, and the shortage of experimental data for comparison.

Using the Vriens based cross section for p-CH_4 results for the total number of ion pairs via primary events only, have been calculated and seem encouraging. Similar results for Gryzinski model are awaited from the university main computer. Estimates of secondary and tertiary events have been made using Vriens, Gryzinski and Gerjuoy-Vriens indicating by comparison with each other and limited experimental data that they are in relatively close agreement. The Gerjuoy - Vriens method offers some computational advantages.

For stopping cross sections the methods of Lindhard et al, and Firsov have been used and results compared with experimental data for

$$p \longrightarrow CH_4 , C_2H_2, C_2H_4 , \text{ and}$$

$$He \longrightarrow CH_4 , C_2H_2, C_2H_4 ,$$

$$N \longrightarrow CH_4$$

A need for further measurement of stopping powers in the low energy region is indicated, although Firsov based calculations give encouraging agreement with recent data of Sidenius and Hughes for $N^+ \rightarrow CH_4$ it is felt that Lindhard provides more useful methods in general.

Allowances for alteration in the effective charge of the projectiles because of charge exchange is yet to be introduced to the final range of these calculations.

It is intended that the methods identified as being of value will be used to form a model for estimation of W values on a wider basis of universal projectile-target combination.

Relevance The relevance of the work described above remains that of obtaining data of importance to measurements and estimates of the effect of heavy ion recoils which occur in neutron irradiation of human tissues and it is intended to pursue these studies further beyond the projected termination date.

Title of the project nr. 3 :	Cross-sections for the inactivation of Enzymes by heavy ions (< 100 keV)
Head of project and scientific staff:	Dr. D.E.Watt, H.M.A. Al-Shaibani, A.T. Al-Kazwini and J.W.Cunningham.

Introduction.

Specification of the quantity and quality of a radiation field for purposes of radiation protection is intimately linked with models for the biological action of radiation. Currently the most successful models are the so-called two component (e.g. Katz) and dual-action (e.g. Neary; Rossi and Kellerer) models. These have certain features which, apart from anomalies in survival curve shapes, may be criticised as all are based on energy deposition and take no account of possible different efficiencies of action due to the type of physical interaction involved.

Direct and indirect action are not treated explicitly - which is necessary to account properly for the influence of modifying factors (e.g. O_2, water and rate effects). In addition one might surmise reasonably that until we can obtain a model which can satisfactorily account for the mechanisms of the action of radiation on enzymes in the dry state and in solution i.e. relatively simple systems in biomolecular/biological terms, then it is very unlikely that we will be able to derive a model which adequately describes the radiation action in the very considerably more complex cellular systems. Such thinking inevitably queries the value of absorbed dose for the specification of radiation quantity and the use of quality factor (and RBE's) for the specification of quality. The value of the energy-based microdose parameters is similarly queried.

To explore these ideas cross-sections for inactivation of enzymes, in the dry state under vacuum, by low energy heavy charged particles (H, He and N ions < 10 keV) were measured. The particle types were selected because of their relevance to intermediate energy neutron irradiation of tissue and the energy region was chosen because the LET of the particles is approximately constant whereas the dominant type of physical interaction changes progressively from inelastic to elastic with decreasing energy and the total number of collisions of all types associated with a single primary target changes rapidly (Fig. 3.1). Also these ions are expected to produce widely differing degrees of saturation effects. We wished to know (a) whether the inactivation cross-section as a function of incident particle energy follows more closely to the trend with LET or to the trend with frequency of events and (b) if the efficiency of the radiation action depends on the type of basic physical interaction involved

Experimental Results.

In principle, the cross-sections for inactivation of ribonuclease were /

were deduced directly from the measured survival curves. However there were severe practical difficulties to be overcome because the range of the charged particles at energies < 10 keV is only a few $\mu g/cm^2$. (A 100 eV proton barely crosses a single RNase molecule!). Consequently true track-segment experiments were impossible at low energy and so we elected to perform thick target measurements in which the incident particle was stopped in the enzyme layer leaving an indestructible fraction. Technical difficulties included: obtaining a thin uniform layer of enzyme which tended to be deposited into crystalline islands about 25 $\mu g/cm^2$ thick i.e. about 50to 100times the nominal thickness; determination of the indestructible fraction of enzyme as there was a highly skewed distribution of particle penetration depths due to straggling and channelling. How these problems were overcome is described fully in the references (2, 4, 8).

The experimental survival data were analysed by computer on the basis of a least squares fit to the equation

$$F - F_o = (1 - F_o) \exp(-\varphi.S) \qquad (1)$$

where φ is the incident particle fluence, F_o is the indestructible fraction, F is the surviving fraction and S is the experimental inactivation cross-section. S corresponds to the differential cross-section averaged over the mean penetration depth of the incident particle.(2) From the theoretical analysis in references (2) and (7) it can be seen that the true differential inactivation cross-section can be obtained from the initial slope of the survival curve. This was demonstrated more specifically by Sigmund and Johnson who gave the differential inactivation cross-section $\sigma(o)$ as

$$\sigma(o) = \left|\frac{dE}{dx}\right| . X . \left[\frac{\partial B_o}{\partial E}\right] \qquad (2)$$

where X is the total thickness of the enzyme layer expressed in mass per unit area and $B_o = \left(\frac{\partial F}{\partial \varphi}\right)_{\varphi = 0}$, is the slope of the survival curve at zero fluence.(6) Careful computerised curve-fitting involving statistical tests was used to minimise errors associated with the differentiation in equation (2). B_o is related to S, through the relationship $B_o = S.\frac{\bar{R}}{X}$ where $\bar{R}$ is the mean penetration depth.

Knowledge of $\sigma(o)$ enables the detailed spatial distribution of damage to be determined whereas knowledge of S enables only the gross damage to be calculated. Both quantities are useful.

In Table 3.1 are presented the most recent experimental results for $\sigma(o)$ and S as a function of particle energy for protons, helium and nitrogen ions in ribonuclease. These are shown graphically in Fig. 3.2 where it can be seen on comparison with Fig. 3.1 that the trend of cross-section with particle energy strongly favours the frequency of events rather than energy deposition as playing the dominant role in the radiation action.

Other important facts which can be deduced from the data are the mean energy (W) expended to inactivate an enzyme molecule, and the threshold energy of the incident particle for inactivation by inelastic collisions.

W/

TABLE 3.1

Cross-sections and W values for inactivation of Ribonuclease.

HYDROGEN				HELIUM			NITROGEN		
E (keV)	$\sigma(Q)$ x 10^{-14} cm^2	S*	W eV	E keV	$\sigma(Q)$ x 10^{-14} cm^2	W eV	E keV	$\sigma(Q)$ x 10^{-14} cm^2	W eV
0.2	6.01 ± .27	35.6	49.3	0.5	13.2 ± .61	79.5	0.145	27.2	97.2
0.3	6.56 ± .31	26.9	51.1	0.6	12.76 ± .61	82.2	0.2	28.8	100.5
0.5	6.63 ± .35	13.6	58.5	1.2	10.56 ± .64	99.3	0.5	32.8	112.6
1.0	5.6 ± .41	12.3	81.4	2.1	7.52 ± .75	139.5	1.0	35.2	123.1
1.5	4.53 ± .55	9.8	120.8	2.8	5.56 ± .88	192.7	1.5	36.5	126.8
2.0	2.9 ± .66	11.6	208.3	4.0	3.04 ± 1.26	356.3	2.0	37.2	129.3
3.0	2.86 ± .69	16.1	241.5	6.0	1.45 ± 1.97	817.6	3.0	38.2	131.0
4.0	3.08 ± .87	11.3	255.4	8.0	3.83 ± 2.91	333.4	5.0	38.3	131.0
5.0	3.58 ± .97	12.8		9.0	6.77 ± 3.55	195.3	7.0	38.2	128.3
7.0	5.86 ± 2.24	11.4	173.5	10.0	10.98 ± 4.22	124.6	8.0	38.3	126.8
8.0	7.66 ± 3.52	8.3	139.9				9.0	38.4	124.7

* Errors on S are about 30%.

Fig. 3.1 Stopping power (L_∞) and the total mean number of collisions (F) associated with interactions in a primary target for helium ions < 10 keV.

TABLE 3.2

Theoretical Cross-section ratios for ribonuclease.

E (keV)	HYDROGEN σ_B/σ_g	E (keV)	HELIUM σ_B/σ_g	E keV	NITROGEN σ_B/σ_g
0.2	0.55	0.5	0.93	0.5	3.15
0.3	0.48	0.6	0.95	1.0	3.76
0.5	0.39	1.2	0.85	1.5	4.0
1.0	0.31	2.1	0.73	2.0	4.1
1.5	0.36	2.8	0.68	3.0	4.1
2.0	0.25	4.0	0.62	5.0	4.0
3.0	0.32	6.0	0.53	7.0	3.98
4.0	0.42	8.0	0.52	8.0	3.95
5.0	0.54	9.0	0.54	9.0	3.92
7.0	0.77	10.0	0.57	10.0	3.94
8.0	0.86				

Errors on the cross-section ratios could be about 30%.

Fig. 3.2 Cross-section ratios for inactivation of RNase for H^+ and He^+ as a function of particle energy.

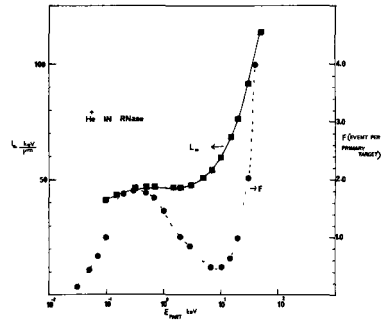
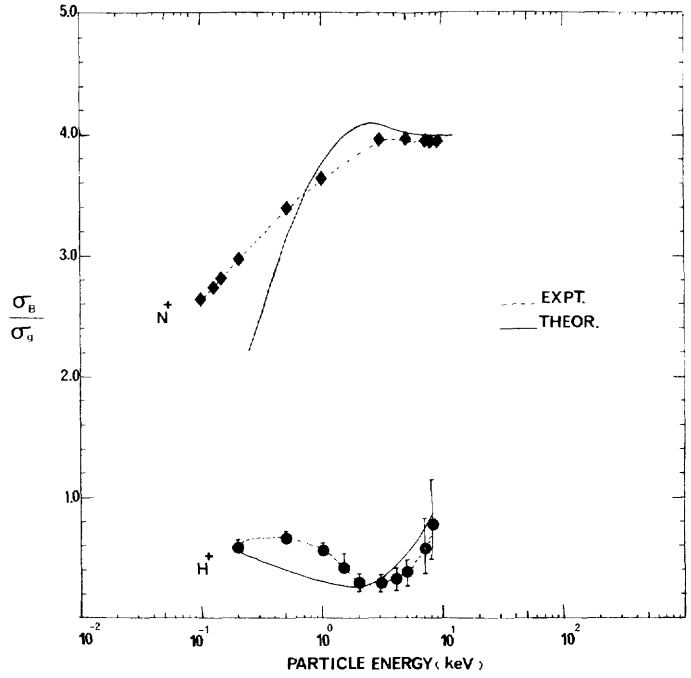
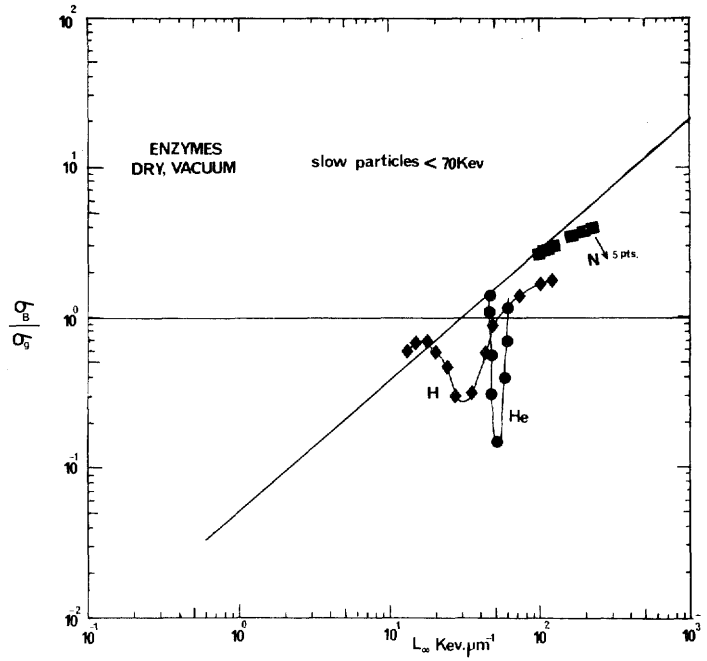


Fig. 3.3 Cross-section ratios for inactivation of enzymes. Data for fast particles in various enzymes is represented by the diagonal solid line. Data for slow H, He and N ions in RNase are represented by experimental points. Note the rapid change of cross-section for He although the L_∞ is approximately constant near 50 keV/μm.


5.0
4.0
3.0
2.0
1.0
$\frac{\sigma_B}{\sigma_g}$
N+
H+
EXPT.
THEOR.
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
PARTICLE ENERGY (keV)


ENZYMES
DRY, VACUUM
slow particles < 70Kev
$\frac{\sigma_B}{\sigma_g}$
N
5 pts.
H
He
10^{2}
10^{1}
10^{0}
10^{-1}
10^{-2}
10^{-1}
10^{0}
10^{1}
10^{2}
10^{3}
L_∞ Kev.μm^{-1}

$W, (= \frac{L_\infty}{\sigma(0)} \cdot v)$ where v is the volume of the molecule and L_∞ is the total stopping power, reflects the efficiency of action of the radiation. Reference to Table 3.1 shows that W is not constant as assumed implicitly in most descriptions of radiation action and also that inactivation by elastic collisions (e.g. protons < 1 keV) can be up to four times more efficient than damage by inelastic (electronic) collisions (e.g. protons > 1 keV) for equal L_∞ . For protons in this energy region there is no energy wastage due to saturation. On the other hand, when compared with inactivation data for fast charged particles, it is found that the inelastic events in the low energy region are significantly less efficient than the inelastic events at higher energies due to the greatly reduced intensity of δ-rays at energies below the Bragg peak. These conclusions are compatible if the radiation action is described in terms of numbers of events rather than energy deposition.

Estimates of the mean threshold energies for inactivation by electronic collisions can be obtained by plotting the modified fraction $1 - \exp(-\sigma\varphi)$ of enzyme against primary particle energy for a range of fluences. For protons, the threshold is about 3 keV which corresponds to an average energy transfer of about 6 eV to a single electron suggesting that rupture of a single molecular bond is a sufficient criterion for the onset of single hit inactivation.[6]

Theoretical Developments.

In parallel with the experimental programme, theoretical calculations of the inactivation cross-sections were pursued. A new model of radiation action was evolved as described in references 1-5 and 9. This model is based on the single hit - single target concept involving numbers of interactions and separates direct and indirect effects. It has sufficient flexibility to cope with the modifying action of changes in the chemical environment (e.g. oxygen, water); saturation effects and spatial effects. The effect cross-section is given[39] by

$$\frac{\sigma_B}{\sigma_g} = \left[P\left(\sum_j h_{i,j}\right) + \sum_j h_{i,j} \cdot n_j \cdot P(h_j) \right] + \sum_j \varepsilon N_j \qquad (3)$$

Here P(h) indicates the probability of survival for a mean number of hits h. i represents the incident particle and j the secondary particle produced in a collision. n_j is the number of secondary targets penetrated by the secondary and ε is the chemical efficiency associated with the total number of collisions N_j. Equation (3) was evaluated using basic physical collision theory.[2] Results are seen to be generally consistent with experiment, (Fig. 3.2) bearing in mind the uncertainties in the physical theory. The theoretical cross-section ratios (equation 3) are listed in Table 3.2.

From this work it is deduced that the physical parameters which may best be used to quantify the radiation field are the combined fluence φ and the geometric cross-sectional area of the biological target, σ_g, rather than absorbed dose. For fast ions quality is specified by z^{*2}, β_i^2, $\bar{d}$ (the mean chord length through the geometrical target) and ε_δ (the efficiency of chemical action). For slow ions, the quality parameters are 't_m' (the Lindhard reduced energy parameter[2]), $\bar{d}$ and ε.

For /

For fast ions, equation (2) can be simplified to the form

$$\frac{\sigma_B}{\sigma_g} = A \cdot Q - BQ^2 \qquad (4)$$

where A and B are constants and the quality parameter $Q = \bar{R}_\delta \cdot z^{*2}/\beta_i^2$. $\bar{R}_\delta$ is the energy-weighted mean range of the δ-rays. Change of ε due to air or aqueous solution modifies the magnitudes of the constants in equation (4). Work is continuing in an attempt to extend the model to more complex organisms using a phenomenological approach supported by new experiments.

Our original intention was to extend these cross-section measurements to 100 keV and for other enzymes. The new 100 kV accelerator has been commissioned and results from this are expected in 1981. Otherwise all original objectives have been attained. Further experiments are planned, with soft X-rays, for enzymes in aqueous solution.

The consequences of this work for radiation protection could be significant. It demonstrates explicitly the inadequacy of parameters based on absorbed energy for specifying radiation action particularly when the dominant type of physical interaction involved is other than ionization e.g. for intermediate energy neutrons (< 20 keV), Auger electron cascades; at tissue interfaces near ingested alpha emitters.

Microdosimetry is demonstrated to be invalid in the present context (e.g. see He results in Fig. 3.3).

Perhaps the most significant feature of this work is that it pioneers the development of a model for the biological action of radiation which can circumvent the difficulties experienced with the current models based on energy deposition and enable more reliable extrapolation to low doses.

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1) WATT, D.E. and SUTCLIFFE, F.J. 'Atomic recoils, quasi-elastic scattering and Radiation Dosimetry'. Second Symposium on Neutron Dosimetry in Biol. and Med. EUR 5273, 1270141, 1975.

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11) TWAIJ, D.A.A. 'Studies in the inactivation of enzymes by radiation in the context of target theory'. M.Sc. Thesis, University of Dundee, December, 1979.

Contractant de la Commission

COMMISSARIAT A L'ENERGIE ATOMIQUE

Centre d'Etudes Nucléaires de Grenoble

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38041 GRENOBLE CEDEX FRANCE

N° du Contrat

178.77.1 - BIO.F.

Chef du Groupe de recherche : Monsieur de CHOUDENS

Chef du Service de Protection contre les Rayonnements

Centre d'Etudes Nucléaires de Grenoble

Thème Général du Contrat : Microdosimétrie

Etude des spectres des transferts linéiques d'énergie et des tailles d'évènement dans les champs mixtes neutron-gamma.

Titre du Projet

Méthode permettant la discrimination des composantes neutron-gamma dans un champ mixte n-γ.

Chef du Projet et Collaborateurs scientifiques

Madame Y. HERBAUT

Monsieur J.B. LEROUX

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0 - INTRODUCTION

Dans les situations pratiques, une fluence neutronique est toujours accompagnée par une fluence photonique. Un système dosimétrique devra donc être capable de séparer quantitativement les doses absorbées des deux composantes d'un champ mixte. La mise au point d'une méthode d'étude dosimétrique de tels faisceaux entraîne des applications importantes tant du point de vue de la radiobiologie pour prévoir les variations de l'efficacité biologique relative dans la pénombre d'un collimateur par exemple, que du point de vue de la radioprotection pour la détermination du facteur de qualité des rayonnements mixtes.

La méthode qui a été étudiée et mise au point est fondée sur la mesure des spectres de tailles d'évènement à l'aide d'un compteur proportionnel type Rossi, rempli d'un gaz équivalent tissu à basse pression et associé à une électronique classique de spectrométrie.

La partie basse des distributions de tailles d'évènement mesurées sous irradiation neutronique correspond à la composante gamma du champ mixte mesuré.

Son adaptation à une distribution semblable obtenue dans le cas d'un rayonnement photonique pur nous a permis de distinguer la contribution due aux électrons de celle due aux particules secondaires mises en mouvement par des neutrons.

Après avoir déterminé les distributions de tailles d'évènement pour plusieurs énergies de rayonnement photonique et notamment pour le ^{60}Co et le ^{24}Na et pour différents diamètres simulés à l'aide du détecteur de type Let 1/2 de la firme Far West Technology, nous avons mesuré les spectres d'ionisation et déduit les courbes de tailles d'évènement pour les configurations d'irradiation neutronique suivantes :

. spectre du réacteur Silène du Centre d'Etudes Nucléaires de Valduc

. neutrons de 14,7 MeV dans l'air, et derrière un collimateur ayant des parois intérieures en acier ou en polyéthylène.

Nous avons pu en déduire à l'aide de la méthode mise en oeuvre :

- le rapport H = Dg/Dn des rayonnements mixtes étudiés (Dg et Dn représentent respectivement les doses absorbées des composantes photonique et neutronique du champ mixte)

- les valeurs des paramètres microdosimétriques $\overrightarrow{y_F}$, $\overrightarrow{yD}$, dans le cas du spectre total ou lorsque sa composante gamma est soustraite

- le facteur de qualité effectif du champ mixte mesuré à partir du pourcentage d'énergie déposée par les différentes catégories de particules secondaires

- à l'aide de la détermination du rapport H par cette technique, nous avons pu en déduire, de plus, la sensibilité relative

neutron-gamma de deux détecteurs que l'on utilise fréquemment dans la méthode dite des chambres associées : le compteur Geiger-Müller et la chambre d'ionisation C/CO_2.

Associé à une électronique non linéaire et intégré dans un système dosimétrique portable, le compteur proportionnel type Rossi devrait donc permettre à la fois la détermination de la dose absorbée et du facteur de qualité effectif. Tel est l'objet des recherches proposées pour la période 1981-1984.

1 - PRINCIPE DE LA METHODE

Une des méthodes de discrimination des contributions en dose des deux composantes d'un champ mixte n-γ est fondée sur la différence qui existe entre le transfert linéique d'énergie pour les électrons et celui des particules secondaires produites par les neutrons.

Un instrument sensible à cette différence est le compteur proportionnel équivalent-tissu type Rossi.

En ajustant, comme l'a suggéré H.G. Menzel (/1/), la partie la plus basse des distributions de tailles d'évènement mesurées sous irradiation neutronique à la distribution de tailles d'évènement obtenue pour un rayonnement photonique pur (le ^{24}Na par exemple), il est possible d'obtenir le rapport $H = \frac{Dg}{Dn}$ par la relation suivante :

$$\frac{Dg}{Dn} = \frac{Ag}{An} \cdot \frac{Wg}{Wn}$$

(Dg et Dn sont les doses absorbées dues aux contributions photonique et neutronique du champ mixte.

Ag et An sont respectivement les aires des distributions photonique ajustée et neutronique ; W_g et W_n sont les énergies moyennes dépensées dans le gaz équivalent-tissu pour chaque paire d'ions créée, lorsque les particules secondaires sont issues respectivement d'une irradiation gamma ou neutron).

2 - SPECTRES DE TAILLES D'EVENEMENT PRODUITS PAR DES RAYONNEMENTS AYANT UN FAIBLE TRANSFERT LINEIQUE D'ENERGIE

Les distributions différentielles f(y) de tailles d'évènement y (y est défini comme le quotient de l'énergie déposée ε par une particule dans un volume V par la corde moyenne $\overline{l}$ de ce volume : $y = \frac{\varepsilon}{\overline{l}}$) sont mesurées à l'aide d'un compteur proportionnel sphérique rempli d'un mélange gazeux équivalent tissu Rossi-Failla à différentes pressions selon le diamètre du site (ds) que l'on désire simuler - Son étalonnage est effectué grâce à une source interne d'^{241}Am. Les spectres sont enregistrés à 3 ou 4 gains électroniques différents, rassemblés et ajustés à l'aide d'un programme de calcul (/2/).

Appelons :

$\overline{y_F}$ le moment du premier ordre de la distribution f(y)

$\overline{y_D}$ le quotient du moment du 2e ordre par $\overline{y_F}$

$$\overline{y_F} = \int_0^\infty y\, f(y)\, dy$$

$$\overline{y_D} = \int_0^\infty y^2\, f(y)\, dy \,.\, \frac{1}{\overline{y_F}}$$

Le tableau 1 rassemble les paramètres microdosimétriques importants des différents rayonnements ($\overline{y_F}$, $\overline{y_D}$) pour le diamètre simulé d_s. La méthode d'extrapolation employée est celle présentée dans la référence /3/.

Le rapport $\overline{y_F}$ (wall-less)/$\overline{y_F}$ (wall) tabulé dans la dernière colonne peut être considéré comme une représentation de l'effet dû à la paroi (/3/) : nous constatons une légère augmentation de cet effet lorsque l'énergie E_γ du rayonnement gamma décroît, moins importante que ne l'indique la référence /3/.

Nous avons reporté la variation des paramètres $\overline{y_F}$ et $\overline{y_D}$ en fonction de E_γ sur la figure 1 : nous observons la transition continue de l'effet photoélectrique à l'effet compton comme processus prédominant de production des électrons dans la région 50-150 keV.

Tableau 1

Eγ (MeV)	ds (μm)	$\overline{y_F}$ (keV μm^{-1})	$\overline{y_D}$ (keV μm^{-1})	$\frac{\sigma^{-2}}{\overline{y_F}^2}$ *	$\frac{\overline{y_F}(WL)}{\overline{y_F}(W)}$ **
2 061 (^{24}Na)	1	0.28	1.78	5.36	0 90
	2	0.25	1.42	4 68	0.89
	3	0.26	1.26	3.85	0 86
1.25 (^{60}Co)	1	0.325	1.915	4.89	0.86
	2	0.30	1 59	4.30	0 86
	3	0.27	1.30	3.81	0 94
0 662 (^{137}Cs)	1	0.43	2 22	4.16	0.86
0.205	1	1.04	3 34	2.21	0.85
0.100	1	1.51	4.34	1 87	0.81
0.060 (^{241}Am)	1	1.37	4.17	2.04	0.83
0 018	1	2.92	5.235	0.79	0.79

* $\frac{\sigma^{-2}}{\overline{y_F}^2} = \frac{\overline{y_D}}{\overline{y_F}} - 1$

** Ces valeurs ont été tirées de la référence /4/.

Tableau 1 - Paramètres microdosimétriques pour différents spectres photoniques

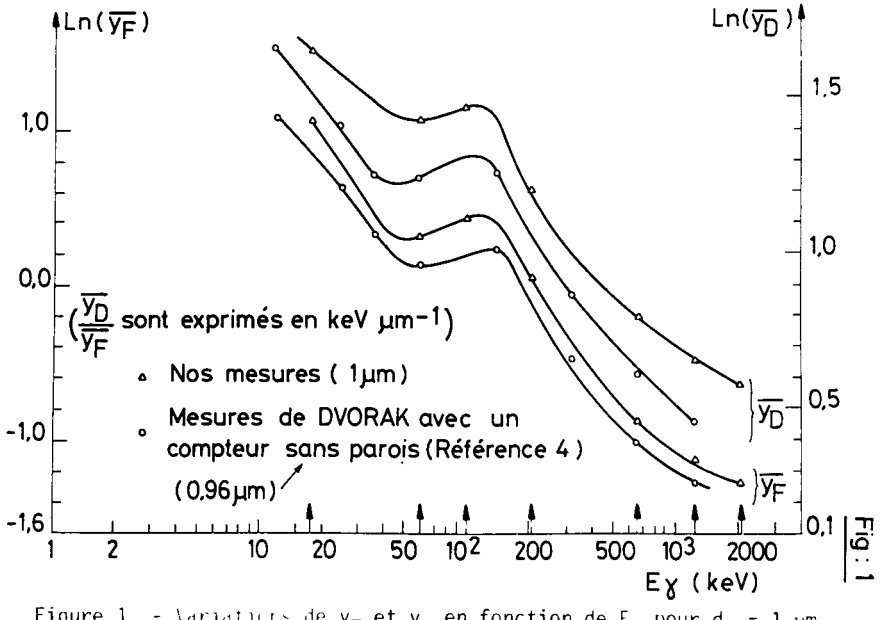

Figure 1 - Variations de y_F et y_D en fonction de E_γ pour d_s = 1 μm

Neutrons 14,7 MeV
Let 1/2 ($d_s = 2\mu m$)

Faisceau	$\overline{Y}_F$ (keV/µm)		$\overline{Y}_D$ (keV/µm)		$\overline{Y}_{F,n}$ (keV/µm)		$\overline{Y}_{D,n}$ (keV/µm)	
	(1)	(2)	(1)	(2)	(1)	(2)	(1)	(2)
free air (au centre)	2,9		97,5		13,3		104,4	
Position 1	3,0	3,7	96,0	97,8	12,5	13,0	103,0	102,7
Position 2	3,1	3,9	99,0	96,0	12,4	12,8	105,3	100,4
Position 3	1,8	2,3	85,8	81,3	14,6	16,4	97,2	89,7
Position 4	1,3	1,6	71,8	63,2	18,9	23,7	87,0	73,9
Position 5	0,9	1,4	45,5	55,0	29,5	25,3	62,7	65,7

(1) Collimateur polyéthylène
(2) Collimateur acier

Tableau 2

Tableau 2 - Paramètres microdosimétriques pour E_n = 14,7 MeV et d_s = 2µm

Fig. 2

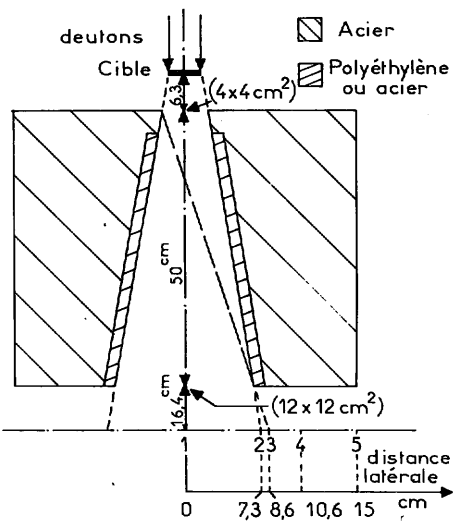

Schéma du collimateur

Figure 2 - Schéma du collimateur utilisé pour les irradiations neutroniques (E_n = 14,7 MeV)

3 - DISTRIBUTIONS DES TAILLES D'EVENEMENT DANS LE CAS D'UNE IRRADIATION NEUTRONIQUE

Nous avons irradié le même dispositif expérimental pour un diamètre simulé de 2 µm par des neutrons de 14,7 MeV, sur l'axe du faisceau et dans la pénombre d'un collimateur (figure 2).

Pour un même collimateur, comme d'autres auteurs l'on déjà mentionné (/5/),nous avons pu constater, lorsque l'on s'écarte de l'axe du faisceau :

. un accroissement des évènements d'énergie linéique inférieure à 1 keV.μm^{-1}

. une diminution de la contribution des évènements dus aux protons de grande énergie

. une diminution de la contribution due aux particules α et aux ions lourds

. une augmentation de la contribution due aux neutrons d'énergie dégradée, dans la région 30-150 keV.μm^{-1}.

Pour une même position latérale et pour des parois intérieures du collimateur de nature différente, la contribution gamma est plus importante dans le cas du polyéthylène, le pourcentage des évènements dus aux protons de faible énergie est plus grand dans le cas de l'acier.

Dans le tableau 2, nous avons rassemblé les valeurs des paramètres $\overrightarrow{y_F}$, $\overrightarrow{y_D}$, en fonction du déplacement latéral du détecteur par rapport à l'axe du faisceau.

($\overrightarrow{y_F}$ et $\overrightarrow{y_D}$ sont relatifs au faisceau total, $\overrightarrow{y_{Fn}}$ et $\overrightarrow{y_{Dn}}$ se rapportent au spectre composante gamma soustraite).

Le facteur de qualité effectif de ces différents champs mixtes a été déterminé de deux manières différentes :

- par différentiation des spectres mesurés ($\overrightarrow{Q_F}$)
- en pondérant les contributions relatives en dose de trois types de particules secondaires (électrons, protons, ions lourds) par le facteur de qualité correspondant ($\overrightarrow{Q_{Fc}}$).

Nous observons une bonne concordance entre ces deux méthodes sur le tableau 3 : la valeur du facteur de qualité au centre du faisceau (7,6) est en bon accord avec d'autres auteurs (/5/).

Tableau 3

Faisceau	$\overline{Q_F}^{(1)}$	$\overline{Q_{Fc}}^{(1)}$	$\overline{Q_F}^{(2)}$	$\overline{Q_{Fc}}^{(2)}$
free air (au centre)	/	7,51	/	/
Position 1	7,53	7,56	7,76	7,72
Position 2	/	7,58	7,66	7,53
Position 3	7,31	7,16	7,80	7,84
Position 4	7,05	6,92	8,03	8,11
Position 5	6,17	6,21	8,03	7,97

(1) Collimateur Polyéthylène
(2) Collimateur Acier
$\overline{Q_F}$: facteur de qualité déterminé à l'aide de la table N C R P

$$\overline{Q_{Fc}} = \frac{Dg}{D_t} + (0{,}8 + 0{,}16\,\overline{L_{Dp}})\,\frac{D_p}{D_t} + 20 \times \frac{Di.l.}{D_t}$$

Neutrons: 14,7 MeV
Utilisation du compteur Let 1/2
$d_s = 2\,\mu m$

Tableau 3 - Facteur de Qualité en divers points de la pénombre du collimateur

Fig. 3

Neutrons 14.7 MeV
Collimateur acier

d_s (µm)	$\overline{Q_{FC}}$
1	8.2
2	7.72
3	7.50
3.5	7.43

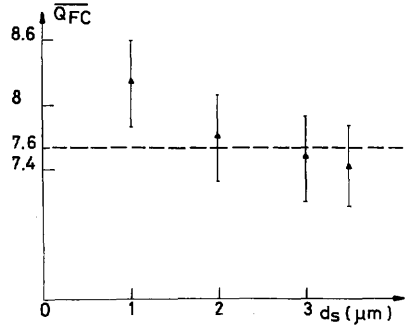

Figure 3 - Variation de Q_{FC} en fonction de d_s pour E_n = 14,7 MeV.

Comme on peut le voir sur la figure 3, $\overline{QF_c}$ diminue légèrement lorsque d_s augmente, en liaison avec la variation de la quantité $\overline{L_{Dp}}$ ($\overline{L_{Dp}}$ est la moyenne en dose du transfert linéique d'énergie pour les protons seuls) qui intervient dans l'expression du tableau 3.

La détermination du rapport H à l'aide du compteur de Rossi permet conjointement avec la technique des chambres associées de déterminer la sensibilité k_U de détecteurs peu sensibles aux neutrons. Pour E_n=14,7MeV, nous avons obtenu pour la chambre C/CO_2 K_u = 0,26 et pour le compteur Geiger-Müller (type Philips 18509) $K_u = 1.3.10^{-2}$.

4 - CONCLUSIONS

La méthode microdosimétrique s'est révélée bien adaptée à l'étude de de la variation de la qualité d'un rayonnement mixte dans la pénombre d'un collimateur, grâce à l'utilisation d'un détecteur de petite taille d'une part, par les informations qu'elle permet d'obtenir d'autre part.

L'interprétation et l'analyse des distributions de tailles d'évènement nous ont permis de tirer des conclusions relatives à la composition des différents rayonnements mixtes étudiés, en particulier la discrimination dose gamma-dose neutron a pu être effectuée.

Utilisé comme dosimètre absolu, le compteur proportionnel type Rossi permet de plus, à l'aide de la connaissance de la contribution relative gamma dans un champ mixte n-γ, de déterminer le facteur de qualité effectif, tout en évitant la méthode mathématique complexe de dérivation des distributions de taille d'évènement. Ce renseignement, nécessaire pour le calcul de l'équivalent de dose, est d'importance pour la radioprotection.

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J.B. Leroux et al - Discrimination des doses dues aux neutrons et aux photons dans un champ mixte n-γ par technique microdosimétrique et chambres d'ionisation - ATSR - Lyon 1980.
Rayonnements Ionisants 4/80.

Contractor	: Radiobiological Institute TNO, Rijswijk, The Netherlands
Contract no.	: 229-76-10 BIO N
Heads of Project	: D.K. Bewley, J.J. Broerse (chairman), G. Burger (vice-chairman), M. Coppola (secretary), J.A.B. Gibson, N. Parmentier, W. Pohlit and G. Portal
General Subject	: Collection and evaluation of neutron dosimetry data (CENDOS)

The results of two international neutron dosimetry intercomparisons, notably the International Neutron Dosimetry Intercomparison (INDI) and the European Neutron Dosimetry Intercomparison Project (ENDIP), indicated considerable differences in the neutron and gamma-ray kerma and absorbed dose values measured by the various groups. Detailed analysis of the calculations performed by the different participants showed a divergency in basic values characterizing the detector response such as the neutron sensitivity of photon dosimeters, the energy required to produce an ion pair in a gaseous detector and the ratio of kerma values in the dosimeter material and in soft tissue.

General agreement on a unique set of parameters will be one of the essential requirements to assure consistency within neutron dosimetry results obtained at different institutes. At the initiative of the Commission of the European Communities a committee had been set up which would be involved with the collection and evaluation of neutron dosimetry data (CENDOS). The specific task of this committee was to review the current status of the field, to compare new results and to encourage and direct new experiments. The scope of these activities was to provide essential information for radiation protection dosimetry and associated radiobiological studies aimed at risk estimates.

During the past contract period the activities of the CENDOS committee have been concentrated on the following coordinated actions:

a. The evaluation of the results of ENDIP was completed. A report of this project has been published (EUR 6004).

b. The experimental results of the three groups participating in the small scale CENDOS intercomparison (notably Fontenay-aux-Roses, Neuherberg and Rijswijk) have been analyzed. The 1977 results for the responses of the ion chambers show a much better agreement that those in 1975. However, none of

the three groups are aware of fundamental changes in experimental techniques applied; consequently, the consistency of the measurement procedures over the two-year period could be questioned. The result of this CENDOS project once again emphasized the need for uniform procedures and techniques for measuring chamber response. In addition to the introduction of a secondary standard or transfer dosimeter, agreement should be reached on the procedures to convert instrument responses into absorbed dose values. The report of this project has been published (EUR 6567).

c. A number of tissue equivalent ionization chambers have been tested and the characteristics of these devices were reported during a workshop on "Ion chambers for neutron dosimetry" organized at the Radiobiological Institute TNO, Rijswijk from September 17-19, 1979. During this workshop the current knowledge on kerma, stopping power, W values and k_U, the relative neutron sensitivity of photon dosimeters, has been summarized. Conclusions were reached on the adoption of a common set of basic physical parameters and on the characteristics of neutron dosimetry systems to be used for on-site dosimetry intercomparisons. The separate contributions have been published in a monograph (EUR 6782).

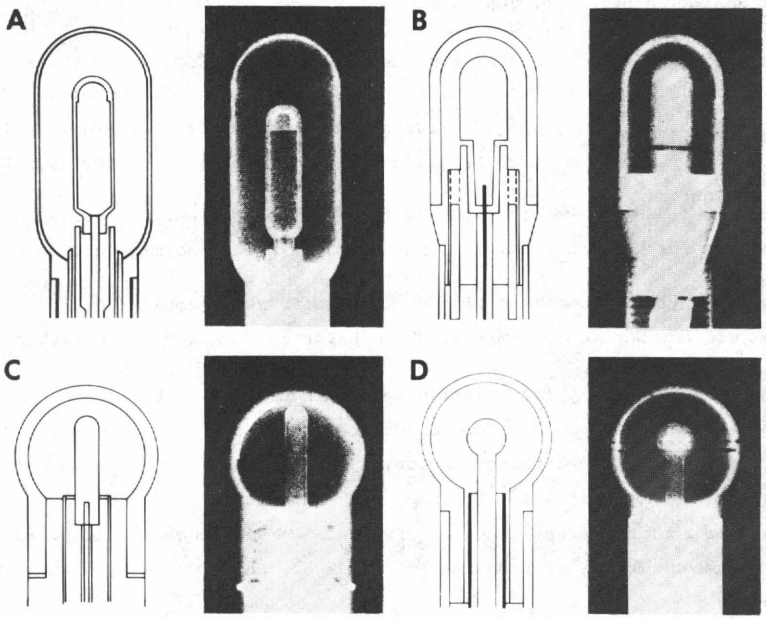
d. In a cooperative study of AVL (Amsterdam), NPL (Teddington), TNO (Rijswijk) and PTB (Braunschweig) the responses of Geiger-Müller counters of different types and with different shields have been determined in the standard d+T neutron fields at NPL (photon component $D_G/D_N = 1.17.10^{-2}$) and at PTB ($D_G/D_N = 0.55.10^{-2}$). The k_U-values derived from the intercomparison are given in the table. It can be concluded that k_U increases with neutron energy and that especially for the higher energies k_U depends on shield design and probably on counter type. For neutron energies up to 5.5 MeV, k_U of the ZP 1100 varies between 0.5 and 1.0 per cent. For d+T neutrons in the energy range between 14.1 and 15.5 MeV k_U values of 1.6 and 2.6 per cent appear to be most realistic for the 18529 counter with Pb/Sn shield and the ZP 1100 Geiger-Müller counters, respectively.

A considerable amount of information has been collected on W values for tissue equivalent gases which could be compiled in a future report. Support has been given to small projects concerning the use of lyoluminescence for neutron dosimetry, the development and testing of a tissue equivalent calorimeter, the calculation of dose equivalent and connected average quality factors in different organs and analysis of dose-effect relationships for carcinogenesis.

TABLE.

Results of cooperative CENDOS study (AVL, NPL, PTB and TNO) on relative neutron sensitivity, k_U, of GM counters with different shield design at different neutron energies.

neutron energy (MeV)	type of counter	composition and dimension of shield	k_U
2.5	ZP 1100	2 mm tin (perforated)	0.49 ± 0.05 (PTB)
5.0	ZP 1100	ibid	0.96 ± 0.08 (PTB)
5.5	ZP 1100	ibid	0.57 ± 0.06 (NPL)
14.7	ZP 1100	ibid	2.3 ± 0.20 (NPL)
15.5	ZP 1100	ibid	2.84 (PTB)
14.1 (collimated)	18529	1.05 mm tin + 0.55 mm lead (perforated)	1.72 ± 0.25 (AVL)
14.7	18529	ibid	1.33 ± 0.20 (NPL)
15.5	18529	ibid	1.71 (PTB)
15.5	18529	1 mm tin	1.48 (PTB)



Design sketches and X-ray photographs of the four tested tissue equivalent ionization chambers manufactured by A: Centre d'Etudes Nucléaires, Fontenay-aux-Roses, CENF, France, B: Exradin, Warrenville, U.S.A., C: Far West Technology, Goleta, U.S.A. and D: Radiobiological Institute TNO, Rijswijk, The Netherlands

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Contractor International Commission on Radiation Units and Measurements

Contract No. 181-76-1 B10 C

Head of the research team : Harold O. Wyckoff

General subject of the contract : Production of recommendations on quantities units, measurements and physical data for clinical radiology, radiobiology, and radiation protection

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Title of the project : Quantities, Units and Measurement Techniques for Ionizing Radiation

Head of Project and Scientific Staff : Harold O. Wyckoff

Pursuant to the contract, the International Commission on Radiation Units and Measurements (ICRU) has continued the development of internationally acceptable recommendations regarding :

1) Quantities and units of radiation and radioactivity,
2) Procedures suitable for the measurement and application of these quantities in clinical radiology and radiobiology,
3) Physical data needed in the application of these procedures, the use of which tends to assure uniformity in reporting.

The Commission has also considered these aspects of the field of radiation protection. In this connection, its work has been carried out in close cooperation with the International Commission on Radiological Protection (ICRP).

The ICRU has continued its efforts to collect and evaluate the latest data and information pertinent to the problems of radiation measurement and dosimetry and to recommend the most acceptable values for current use.

The Commission's recommendations have been kept under continual review in order to keep abreast of the rapidly expanding uses of radiation.

Published

ICRU Report No.	Title and Year of Publication
24	Determination of Absorbed Dose in a Patient Irradiated by Beams of X or Gamma Rays in Radiotherapy Procedures (1976)
25	Conceptual Basis for the Determination of Dose Equivalent (1976)

Published

ICRU Report No.	Title and Year of Publication
26	Neutron Dosimetry for Biology and Medicine (1977)
27	An International Neutron Dosimetry Intercomparison (1978)
28	Basic Aspects of High Energy Particle Interactions and Radiation Dosimetry (1978)
29	Dose Specification for Reporting External Beam Therapy with Photons and Electrons (1978)
30	Quantitative Concepts and Dosimetry in Radiobiology (1979)
31	Average Energy Required to Produce an Ion Pair (1979)
32	Methods of Assessment of Absorbed Dose in Clinical Use of Radionuclides (1979)
33	Radiation Quantities and Units (1980)

Nearing Completion

Reports on the following topics:

(1) radiation dosimetry : electron beams with energies between 1 and 50 MeV
(2) dosimetry of pulsed radiation
(3) computer uses in radiotherapy
(4) microdosimetry
(5) stopping power

Initiated During Contract Period

Work on reports treating the following topics was initiated during the contract period and is now underway:

(1) measurement of low-level radioactivity in humans
(2) definitions and terminology for computed tomography
(3) C_λ and C_E
(4) determination of absorbed dose distribution around a source used for interstitial therapy
(5) practical determination of dose equivalent index
(6) clinical dosimetry for neutrons

(7) modulation transfer function for screen-film systems
(8) absolute and relative dosimetry at high doses
(9) chemical dosimetry
(10) quality assurance of diagnostic radiology equipment
(11) quality assurance of external beam therapy equipment
(12) specification and quality assurance of scintillation cameras
(13) quantities for use in radiation protection

Detailed Report

Objectives: During the contract period the ICRU sought to (1) formulate recommendations on various previously identified topics in radiation measurement and (2) identify additional areas where the development of ICRU recommendations would constitute an important scientific contribution and begin the formulation of recommendations on these topics.

Results: At the start of the contract period the ICRU was engaged in the development of recommendations on many topics. The status of these efforts is indicated below:

Topics	Status
(1) an international neutron dosimetry intercomparison	Published as ICRU Report 27
(2) assessment of absorbed dose in clinical use of radionuclides	Published as ICRU Report 32
(3) basic aspects of high energy particle interactions and radiation dosimetry	Published as ICRU Report 28
(4) average energy required to produce an ion pair	Published as ICRU Report 31
(5) dose specification for reporting	Published as ICRU Report 29
(6) dosimetry of pulsed radiation	Draft before ICRU for review
(7) fundamental quantities and units	Published as ICRU Report 33
(8) photographic dosimetry in external beam therapy	Work underway
(9) radiobiological dosimetry	Published as ICRU Report 30
(10) scanning	Work terminated
(11) visual determination of resolution in screen film system	Work underway
(12) computer uses in radiotherapy	Drafting work nearing completion
(13) microdosimetry	Draft before ICRU for review
(14) stopping power	Drafting work nearing completion

The ICRU continually seeks to identify areas in which the revision of previous recommendations or the formulation of new recommendations would constitute a significant contribution. During the contract period, this process resulted in the initiation of new activities concerned with the development of recommendations on the following topics:

(1) radiation dosimetry : electron beams with energies between 1 and 50 MeV
(2) measurement of low-level radioactivity in humans
(3) definitions and terminology for computed termography
(4) C_λ and C_E
(5) determination of absorbed dose distribution around a source used for interstitial therapy
(6) practical determination of dose equivalent index
(7) clinical dosimetry for neutrons
(8) modulation transfer function for screen film systems
(9) absolute and relative dosimetry at high doses
(10) chemical dosimetry
(11) quality assurance of diagnostic radiology equipment
(12) quality assurance of external beam therapy equipment
(13) specification and quality assurance of scintillation cameras
(14) quantities for use in radiation protection

These efforts are in various stages of completion with some nearing completion, some in what might be term a "midway" stage, and others just beginning.

Significance

An accepted set of quantities and units for ionizing radiation is a requirement for effective communication between individuals concerned with radiation matters. Similarly, information on the various radiation measurement techniques which are appropriate for different purposes can be of immense value to all of those involved in programs that require assessment of irradiation.

Evidence of the value of ICRU publications is given by the fact that approximately 28,000 copies of ICRU reports have been distributed to users during the period of the contract.

Contraente della Commissione:

COMITATO NAZIONALE PER L'ENERGIA NUCLEARE

N. del Contratto: 103-76-1 PST I

Capo del Gruppo di ricerca:

G. Busuoli

Tema generale del contratto:

Studies on New Detectors Useful for Dosimetry

Titolo del progetto

Neutron Dosimetry by Etching Track Methods

Capo progetto e collaboratori scientifici:

A.Cavallini, L.Lembo, O.Civolani

The aim of this research project has been to study the use of the etching track detectors for personal neutron dosimetry along two different techniques: detection of albedo neutrons and detection of recoils produced by neutrons. Systems based on fission converters have not been considered due to radiation protection consideration.

Plastic detectors have been used for the albedo technique coupled with an (n, α) converter in order to measure the thermal neutrons reflected by the human body.

The dosimeter, schematically shown in Fig. 1,consists of cellulose nitrate and a converter of natural LiF evaporated on an alluminum base. Later on the system has been improved using commercial cellulose nitrate coated with lithium tetraborate (Kodak Film CA-80 15 Type B). This albedo dosimeter has a dose equivalent threshold of approximately 50 and 150 mrem for neutron energies of 0.2 and 5.0 MeV rispectively.

However the dosimeter presents a large energy dependence which, even being better than the one of nuclear emulsions, is a strong limitation for a practical use in personal neutron dosimetry.

The second technique, based on measurements of recoils, has used as etching detectors polycarbonates such as Makrofol E 300 μm thick. These plastics have presented drawbacks due to the presence of a large amount of background tracks as a consequence of both structure damages during the preparation of the polycarbonate and environmental radiation. Therefore it was attempted to reduce this background through either particular thermal treatment or chemical pre-etching performed before the irradiations.

The experimental results have however shown that both techniques produce a large background reduction, but are of no practical application. In fact the effects of the thermal treatment are not reproducible and the chemical pre-etching (solution of C_2H_2OH plus Na OH) strongly reduces the sensibility of the plastic. During the same period two different devices for the electrochemical etching of the Makrofol polycarbonate have been set up (Fig. 2 and 3); in this case too the results have been

largely influenced by the background tracks still present.

Later on a new polymer 1.5 mm thick, commercially named Polygard CR-39 manufactured by Polytech (USA), has been investigated. This plastic is provided with both sides coated by a thin plastic film, sufficient to eliminate the tracks due to the background radiation.

The CR-39 dosimeter uses as converter a sheet of poliethylene and has been investigated using a chemical etching in a solution of NaOH-6N at a temperature of 70°C. The energy dependence of the dosimeter, shown in Fig.4, is within a factor of 3 in the range 0.2 MeV to 14 MeV and can be considered small enough for the practical use of the dosimeter.

Using the above chemical etching the background tracks are (170 $\pm$ 30) cm^{-2}; this brings to a dose equivalent threshold, taken as three times the standard deviation of the background, of the order of 10 mrem for Cf-252 neutrons.

The above results show that the CR-39 dosimeter is usable in practice for personal neutron dosimetry and an experimental program has already been planned in order to check its reliability on a routine basis in comparison with nuclear emulsion dosimeters.

However it is essential to continue the research work both for a better characterization of the technique and for studying the electrochemical etching on thinner CR-39 detectors.

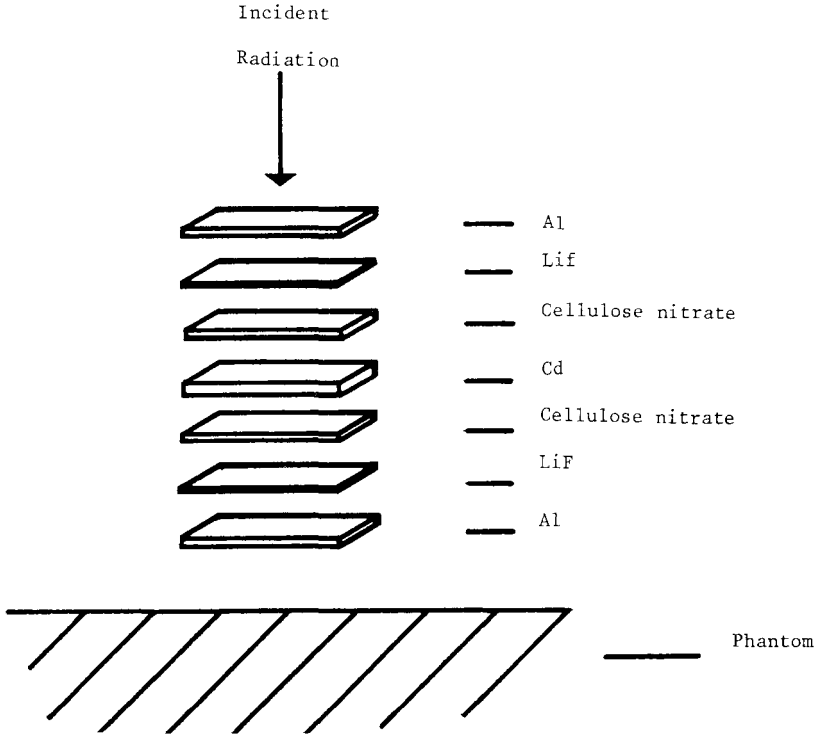

Fig. 1 - Albedo dosimeter with etching track detectors

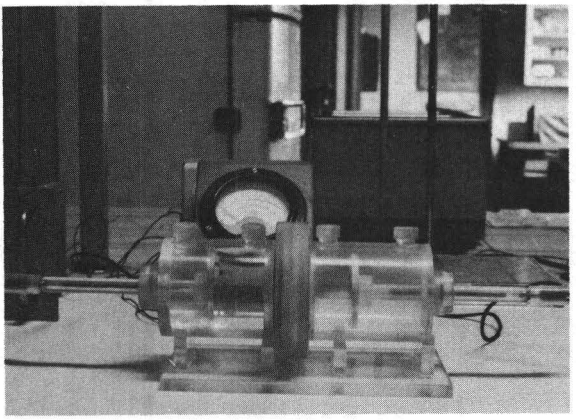

Fig. 2 - Prototype of electrochemical etching device

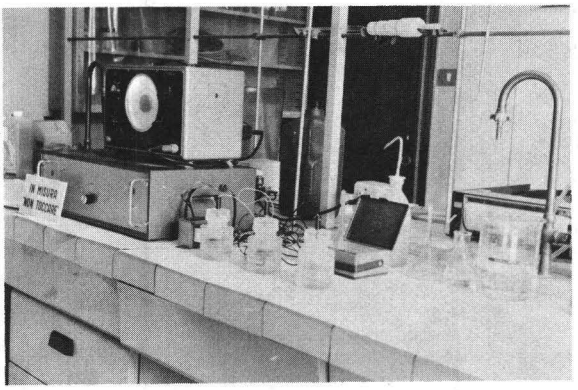

Fig. 3 - Improved prototype of electrochemical etching device

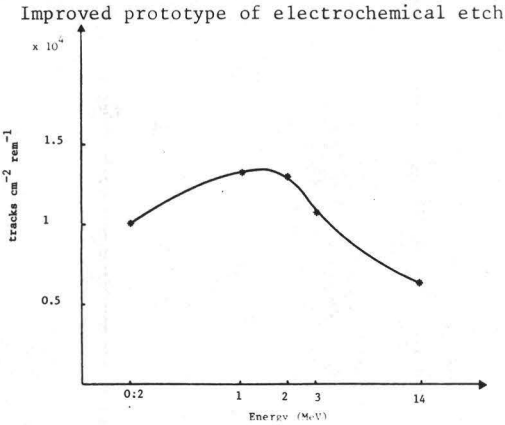

Fig. 4 - Energy dependence of CR-39 dosimeter, chemically etched.

Vertragspartner der Kommission:	Gesellschaft für Strahlen- und Umweltforschung mbH München, Institut für Strahlenschutz
Vertrag Nr.:	106-76-1-PSTD
Leiter der Forschungsgruppe:	Dr. G. Burger, Prof. Dr. W. Jacobi
Allgemeines Thema des Vertrages:	Neutronendosimetrie im Strahlenschutz

The contractors research group "Radiation Physics and Neutron Dosimetry" in the GSF Institute for Radiation Protection is predominantly engaged in radiation protection research.

The long term programme of the Laboratory for Neutron Measurement and Technology in the group covers three main topics:

- the development and application of installations, instrumentation and methods to provide standard neutron fields and detectors for performing calibrations, reference measurements and intercomparison studies.
- the development and improvement of applied techniques for neutron dosimetry and spectrometry.
- the performance of radiation transport calculations.

All these activities were items of the proposal and hence supported to different extents by the contract. With respect to the new international dosimetric concepts, that have been introduced, the laboratory concentrated mainly, in the past two years, on the third project. Several numerical methods were applied to calculate the organ doses in inhomogeneous phantoms as well as the index quantities for the ICRU-sphere.

Aside from this, emphasis was given to calibration and intercomparison studies and, finally, to the further development of personnel dosimeter systems. The working place analysis of occupational neutron exposed people could however not be done to the desired extent.
The contract covered a 12 man-month support per year, about 50 % of which was spent for the intercomparison studies and 50 % for the radiation transport calculations.

1. Calibration facilities, reference fields and intercomparison studies.

During the contract period the neutron calibration facilities of the GSF were completed and used for the postal neutron personnel dosemeter intercomparison project ENMIP (European Neutron Monitorring Intercomparison Project), being held in 1976 and 1977. More than 500 dosemeters from 15 different institutions were exposed and the data were analysed in correspondence with a detailed enquiry on the systems used and the data evaluation applied. The results show clearly the expected shortcomings of the different dosemeter types and were by no means satisfying. They indicate clearly that such intercomparisons should be repeated more frequently, offering not only monoenergetic "calibration radiation" but rather realistic stray and leakage fields, and laying emphasis on exposures below 100 mrem rather than above this value /1, 2, 3/.

In addition a small international intercomparison of track etch detectors for neutron fluence measurements was organized at the end of 1976 /4/. The group participated also in the CEC-sponsored neutron monitoring workshop at the PTB-Braunschweig. The instrumentation used was especially selected with respect to the intercomparison of reference instrumentation responses. The laboratory participated further in an intercomparison of nuclear accidental dosimetry in Oak Ridge, Tennessee, mainly to improve its knowledge about the albedo dosimeter. The existing monoenergetic reference fields at the 3 MeV-Van-De-Graaff-accelerator were complemented by leakage fields from a Cf-252 source /5/ being positioned inside spherical water tanks of various sizes.

2. Neutron dosemeter development and application.

The GSF-albedo type dosemeter /6, 7/ was further checked and used for the analysis of fields inside reactors. For three reactors, one swimming pool research type and two power stations, various personnel and areal detectors were used in extended field studies. The result showed clearly the shortcomings of the NTA-film and the necessity of calibration of the Albedo-system at the monitored working places /8, 9/. The new track etch detector CR-39 was thoroughly investigated with respect to angular and energy dependence of its

response as a function of etching and pre-etching parameters. Conventional as well as electrochemical etching was applied. The investigations will result in a combined new track etch plus albedo type personnel monitor /10/.

3. Radiation transport calculations

Much effort was put into the further development and application of codes for the simulation of radiation transport within materials. The following codes were implemented, tested and the cross section libraries updated:

- The SN codes ANISN (mainly for the generation of group cross sections) and DOT version II and III-B for application in two dimensional geometries.
- The Monte-Carlo-Code SAM-CE for the application in three dimensional geometries as with anthropomorphic phantoms.
- The CHORD-program for the approximate determination of organ doses in anthropomorphic phantoms.

Both the CHORD-method and the Monte-Carlo-Code were applied with the MIRD-phantom and organ doses were determined. The cross sections for the MC-code for the 50 organs with 21 different element compositions were determined from ENDF-B IV. The primary results were mean spectral neutron fluences in the organs. They can be converted into KERMA as well as dose equivalent by means of prepared conversion functions based upon known kerma tables, recoil particle distributions and QF(L)-relationships /11, 12, 13, 14, 15/.

Both the DOT and the SAM-CE code were applied to the homogeneous 30-cm-ICRU-sphere. The axial depth dose distributions and hence the index quantities agree well with each other and with the results of Chen and Chilton /16/. A superposition program was developed, generating the isodose distributions for widening space angles and determining the so called anisotropy factors for the nonadditive index quantities.

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loc. cit. /4/

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The Calculation of Index Quantities by Means of the DOT and the SAM-CE Codes.
loc. cit. /4/

Contractant de la Commission : Association pour le Développement de la Physique Atomique
118, route de Narbonne
31062 TOULOUSE CEDEX

Centre de Physique Atomique
Université Paul Sabatier

N° du contrat : 102 76 1 PST F

Chef du groupe de recherche : Monsieur le Professeur D. BLANC

Thème général du contrat : Réalisation et étude de chambres d'ionisation constituées de diélectriques solides, destinées à la détection des rayonnements électromagnétiques et neutroniques.

Realisation and study about dielectric solids ionization chambers for X rays and neutrons detection.

Description générale.

Nous avons étudié les caractéristiques électriques de différents diélectriques solides organiques et inorganiques sous rayonnements électromagnétiques et neutroniques. En particulier nous avons fait l'analyse systématique du courant induit en fonction des paramètres, champs électrique appliqué, température de l'échantillon et débits d'irradiation, l'application étant essentiellement la réalisation d'un débitmètre.

We have studied induced current in mineral and organic dielectric solids from electromagnetic rays and neutron beams versus dose rate, electric field magnitude and temperature.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques : J. BARTHE, Th. BARTHE

Titre du projet : Réalisation et étude de chambres d'ionisation constituées de diélectriques solides, destinées à la détection des rayonnements électromagnétiques et neutroniques.

Les caractéristiques électriques des structures vitreuses ne sont pas d'une grande sensibilité aux rayonnements ionisants, en particulier la résistivité. Ainsi, il faut des doses déposées supérieures à 20 Gy pour relever une variation de la résistivité dans les échantillons de silice. Cette variation met en évidence l'existence de défauts, ou centres, dont les propriétés électroniques sont bien exploitées dans le domaine des faibles doses, comme par exemple celles de la radiophotoluminescence des verres aux phosphates activés à l'argent.

En débitmétrie la silice pure se révèle comme le matériau le plus sensible.

La conductivité induite permet des mesures instantanées des débits de dose dans la gamme intermédiaire entre les faibles débits ($>$ 1 cGy/h) et les débits très élevés ($<$ 10^3 Gy/h).

Avec un échantillon standard (S = 5 cm^2, e = 0, 2 cm) le débit minimal correctement mesurable est égale à 1 cGy/h.

La réponse de la silice ou rayonnement γ du ^{60}Co est proportionnelle au débit de dose jusqu'à plus 10^3 Gy/h, de la température ambiante à 150°C. Elle dépend peu de la température et possède une énergie d'activation de quelques dizaines de milliélectronvolts.

La réponse sous irradiation, déduction faite du bruit de fond, ne varie pas au cours du temps. Certaines expériences ont été réalisées sur

une période de trois ans avec des lots différents d'échantillons. La conductivité électrique naturelle de la silice peut varier de plusieurs ordres de grandeur suivant sa pureté chimique. Il est donc essentiel de choisir celle qui comporte le moins d'impuretés métalliques.

L'effet de dose intégrée est particulièrement significatif sur les échantillons de silice contenant de l'eau. Il en résulte une diminution de la résistivité naturelle qui est fonction de la quantité d'impuretés dans l'échantillon, mais l'effet de débit de dose reste inchangé.

Nous considérons comme résolus les problèmes technologiques relatifs à la détection des rayonnements électromagnétiques de haute énergie avec des cellules à géométrie plane. Nous avons, parallèlement, effectué les mêmes expériences avec des neutrons rapides dans des conditions analogues.

La réponse de la silice aux neutrons ($\overline{E} = 3,3$ MeV) est sensiblement proportionnelle aux débits de fluence ($\phi \lesssim 15.10^5$ n°/cm^2/s) pour des champs électriques supérieurs à 10 kV/cm. Comme avec le rayonnement γ du ^{60}Co le courant induit est indépendant de l'épaisseur de l'échantillon ($e \gtrsim 500$ µm).

Il n'a pas été possible d'observer une éventuelle saturation en fonction du champ électrique, comme dans le polyéthylène-téréphtalate (Ma-1). Avec les débits utilisés la mesure du courant induit dans des couches minces de silice (épaisseur de quelques micromètres) déposées par pulvérisation cathodique HF est impossible, étant donné la surface réduite de l'électrode collectrice (Sc $\neq$ 0, 14 cm^2) de telles structures. Avec le rayonnement électromagnétique, la valeur absolue du courant induit est indépendante de la polarité du champ électrique de collecte. Par contre, sous flux neutronique, son amplitude est fonction du signe du champ électrique extérieur. Le résultat des expériences faites avec le flux neutronique implique des phénomènes de génération de charges spécifiques.

L'analyse des variations de la conductivité induite dans la silice avec le champ électrique et la température suggère un mécanisme de dis-

sociation des paires électron-trou créées qui obéit à la théorie de diffusion d'ONSAGER. Ce mécanisme est particulièrement bien mis en évidence dans les irradiations avec les rayons γ du ^{60}Co pour des débits de dose relativement faibles (quelques cGy/h à quelques Gy/h). Un résultat intéressant est obtenu dans l'hypothèse d'une fonction de distribution des distances de séparation des paires électron-trou de symétrie sphérique.

L'approximation aux champs faibles de l'expression de la probabilité pour qu'une paire électron-trou dont la distance de séparation initiale est r, échappe à la recombinaison initiale, prévoit une relation linéaire avec le champ électrique E de coefficient $p = e^3 / 8\pi \varepsilon_o \varepsilon_r k^2 T^2$

($p = 2,9.10^{-5}$ cm/V à 298°K avec $\varepsilon_r = 3,8$ pour la silice).

Nous avons étudié plus particulièrement le rendement expérimental Y à partir des mesures de la conductivité induite (σ_i) et de la dose absorbée dans l'échantillon (ξ) rapportée à 100 eV :

$$Y = \frac{\sigma_i}{e} \quad \frac{100}{\xi} \ (cm^2/V)$$

Nous avons démontré que la relation $Y(E,T) = Y(0,T)(1 + pE)$ est vérifiée avec une bonne approximation dans l'intervalle de champ électrique ($0 \rightarrow 3.10^4$ V cm^{-1}).

En dosimétrie, on considère généralement le rendement G en nombre de paires électron-trou pour une énergie absorbée de 100 eV (ou en Coulomb par Joules, dans le système international).

Nous avons donc poursuivi cette étude, connaissant par ailleurs la durée de vie τ et la mobilité μ de l'électron dans la silice (Hu-1) :

$$G(E,T) = Y(E,T) \ / \tau \cdot \mu(T).$$

Avec l'hypothèse d'une distribution isotrope de paires thermalisées, dans le cadre de l'approximation aux champs faibles on obtient la relation :

$$G(E,T) = G(0,T)(1 + pE) \quad \text{où} \quad G(0,T) = G.\exp(-E_a / kT)$$

avec l'énergie d'activation $E_a = e^2 / 4\pi \varepsilon_r \varepsilon_o r_o$ et $G = 100 / W$.

Nous avons pu ainsi faire une estimation de W énergie nécessaire pour créer une paire électron-trou dans la silice ; nous obtenons (16± 2) eV, en accord avec celle déduite de la théorie des plasmons de Rothwarf (Ro-1).

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Contractor: United Kingdom Atomic Energy Authority
Atomic Energy Research Establishment
Harwell, Oxfordshire, OX11 ORA, U.K.

Contract No: 098-76-1 PST UK
Head of research team: Dr D H Peirson

General subject of the contract. DOSIMETRY OF EXTERNAL RADIATION FIELDS: DEVELOPMENT OF MONITORING SYSTEMS.

Title of the project nr 1: Design of Dosimetry Systems for Neutron Monitoring.
Head of Project and scientific staff: J A B Gibson, K G Harrison, A J Taylor, S J Boot, P M Thomas.

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1. Introduction

The aim is to provide a complete neutron monitoring system for use in nuclear-fuel processing plants and other facilities handling high burn-up fuel producing significant numbers of neutrons. The essential feature of the design is to provide an integrated system in which the parts are used to control and measure the dose equivalent to personnel handling fissionable and alpha-active materials. The system consists of five major parts.

(i) personnel dosimeters for recording the dose equivalent over periods up to 1 month.
(ii) pocket alarm neutron dosimeters to provide a warning when a dose equivalent limit is exceeded in any period of 16h.
(iii) survey instruments to establish and check changes in bench-mark conditions.
(iv) installed instruments to warn of sudden changes in the dose equivalent rate.
(v) neutron spectrometry techniques to define the degree of complexity required in the other parts of the system.

In addition it was necessary to make some theoretical calculations of albedo spectra and the response of some detectors including a new phantom design. Calibrations and tests in processing plants and at European intercomparisons were made as part of this project.

2. Personnel Dosimeters for Recording the Neutron Dose Equivalent

It has been found that the neutron spectrum in processing plants is highly variable and so dosimeters relying upon the albedo principle alone would not be adequate. The dosimeter chosen was based upon (n,fission) reactions in 4 mg of Np-237. The fission fragments cause damage tracks in a polycarbonate foil which after removal from the dosimeter is etched in hot potassium hydroxide solution and counted in a semi-automatic spark counter. The theoretical response to a fission neutron spectrum is 40 tracks/mSv (400 tracks/rem) which is reduced to 18 tracks/mSv (180 tracks/rem) in the practical dosimeter. The dosimeter responds to thermal and intermediate-energy neutrons and, when worn on the body, measures albedo neutrons and so gives a dose equivalent response which is within ± 30% for all the neutron spectra found in processing plants. The background is about 6 tracks per year or 340 μSv/yr (34 mrem/yr). There is a small gamma-ray dose to the wearer from the dosimeter but this can be reduced to an acceptable level by shielding.

400 neptunium dosimeters have been manufactured and are in use in various plants in the UK. They are worn on a belt with two albedo dosimeters (Harvey et al, 1973) to provide some indication of the neutron spectrum. (A single chip of ^{6}LiF is used in the albedo dosimeter to give both gamma-ray and neutron data by measuring the light output at temperatures of 210 and 260°C.) The system was tested at the ENMIP Intercomparison and compared favourably with all existing dosimeters.

3. Pocket Alarm Neutron Dosimeter (PAND)

This is based upon a low-pressure proportional counter developed at Harwell. Fast neutrons interact with the hydrogenous walls to produce protons which are detected in the counter. Thermal neutrons are detected by the ^{14}N (N,p) ^{14}C reaction and intermediate-energy neutrons are measured by the albedo from the wearer's body. Integrated circuits were developed to reduce the weight (600 g) and eliminate spurious pulses. The dosimeter has a sensitivity of 1 count per μSv (10 counts/mrem), a gamma-ray sensitivity of < 5% of the neutron dose equivalent sensitivity and a spurious counting rate of < 50 counts (50 μSv) in 8 h. The PAND is mounted on a belt with the neptunium and albedo dosimeters and will operate for up to 16 h between charges. Tests in a processing plant showed that it measured the neutron dose equivalent to within ± 30% for all neutron spectra encountered.

4. Survey and Installed Instruments

The existing neutron survey instrument (AERE type 0075) (Leake, 1968) has been redesigned with modern electronics and a digital liquid crystal display (AERE type 0949). Calibrations with mono-energetic neutrons (0.1 to 2 MeV) and an antimony/beryllium source have shown that the instrument over responds (up to a factor of 5) to intermediate energy neutrons but this is of little practical importance when measuring most neutron spectra in processing plants. Measurements at the PTB, Braunschweig during a Euratom Workshop have shown discrepancies between UK and FDR calibrations at 24 keV and these have still to be resolved. An instrument of similar design to the Leak counter can be used as an installed instrument for measuring sudden changes in the neutron dose-equivalent rate.

A second survey instrument with a smaller sphere (diameter 63.5 mm) has been developed to measure the dose equivalent between thermal and 10 keV neutron energies and to locate narrow beams. The ratio of this instrument to the dose equivalent as measured by the Leake detector is used to determine the response of albedo dosimeters and to provide some information on the variations in neutron spectrum in the processing plant.

5. Neutron Spectrometry in the Field

The laboratory-type of spectrometer based upon organic scintillators and ^{3}He counters developed under CEC contract no 167-76-1 BIO UK can be used in processing plants for measuring neutron spectra from a few hundred keV to 15 MeV. For low energies, a multisphere sphere system (Bramblett et al, 1960) has been developed. Spheres of diameter from 50 to 250 mm have been used with a LiI scintillator and more recently with a ^{3}He proportional counter to reduce the gamma-ray sensitivity, improve the neutron sensitivity and to provide a better isotropic response. Developments in the unfolding techniques to produce the neutron spectra have shown that, in general, the uncertainty in the dose equivalent spectrum is ten times the counting uncertainty thus, for example, at least 10^{4} counts are required for 10% precision. Further developments to produce better response functions for the ^{3}He detector are proceeding.

6. Theoretical Studies and the Development of Phantoms

All parts of the personal dosimetry system have some sensitivity to albedo neutrons reflected from the body and it was necessary to include this effect in calculating the response of each dosimeter. A modified version of the computer programs developed under CEC contract 167-76-1 BIO UK has been used to produce a library of albedo spectra from 10 keV to 14.7 MeV.

In order to test neutron dosimetry systems, it is necessary to provide a suitable phantom and a means of measuring the total integrated dose equivalent over periods up to one month. By placing a thermal neutron detector (viz a ^{6}LiF TLD) at the centre of water and polythene cylinders it has been shown by Monte Carlo methods that both requirements can be fulfilled in one device. Ten polythene phantoms of diameter 250 mm and height 230 mm are being produced and will be tested in standard fields and in a processing plant.

7. Conclusion

The main objective has been met in that a complete neutron dosimetry system has been supplied for a nuclear-fuel processing plant. It was tested before installation in another plant and at various European Intercomparisons. For the future, we intend to continue studies on the multisphere system and develop more sensitive methods of personnel dosimetry using the measurement of recoil ions in CR-39 plastic. We will test these systems in the laboratory, at European Intercomparisons and in working situation of processing plants.

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CONTRACTANT DE LA COMMISSION :

COMMISSARIAT A L'ENERGIE ATOMIQUE - CENTRE D'ETUDES NUCLEAIRES DE FONTENAY-AUX-ROSES - FRANCE -

N° DU CONTRAT : 109-77-1 PST F

CHEF DU GROUPE DE RECHERCHE : G.PORTAL - DPR./STEP/STEPD/LID

THEME GENERAL DU CONTRAT : DOSIMETRIE DES NEUTRONS PAR L'UTILISATION DE DETECTEURS A L'ETAT SOLIDE.

DESCRIPTION GENERALE SUCCINCTE DES TRAVAUX -

L'étude a été engagée en 1977, dans deux directions :

1) Technique de l'Albédo -

La méthode consiste à détecter, au moyen de produits thermoluminescents ou de détecteurs solides de traces, les neutrons lents réémis après diffusion et ralentissement dans le corps humain. Les effets enregistrés sont reliés à l'équivalent de dose.

2) Emission exoélectronique thermostimulée -

Dosimétrie des neutrons rapides par la mesure dans un détecteur mince de l'ionisation due aux protons de recul issus d'un radiateur hydrogéné. Ces deux voies sont complémentaires : l'effet d'albédo est prépondérant aux basses énergies; la mesure des protons de recul n'est réalisable qu'aux énergies supérieures à quelques keV.

DESCRIPTION DES RESULTATS -

1) Technique de l'albédo -

Deux voies différentes ont été explorées :

a) La thermoluminescence -

Le départ de l'étude a consisté à essayer un dosimètre, proposé par HARVEY, avec des pastilles thermoluminescentes de FLi fabriquées par DESMARQUEST-CEC : PTL 710 avec du lithium naturel, PTL 717 avec du lithium 7 insensible aux neutrons lents.

Les essais du dosimètre sur fantôme, avec des neutrons monoénergétiques du PTB de Brunswick ont montré que la sensibilité du Li naturel était faible et que la dépendance énergétique était très forte, passant, en relatif, de 100 à 1 entre l'énergie thermique et quelques MeV alors que la variation relative de l'équivalent de dose est de 1 à 35 (voir figure n° 1).

Nous avons réalisé en laboratoire des frittés de PTL 716 contenant du lithium enrichi au lithium 6. L'évaluation de la sensibilité du PTL 716 aux neutrons thermiques a été entreprise auprès de l'empilement Sigma de Cadarache. Le gain de sensibilité du PTL 716 par rapport au PTL 710 est de 3,5 pour les neutrons thermiques. Nous avons mis en oeuvre ces pastilles dans le boîtier PGP1, utilisé en routine pour la dosimétrie γ avec lecture automatique . Il a été équipé de 4 pastilles : 2 PTL 717, 1 PTL 710 et 1 PTL 716 (voir figure n° 2). Le boîtier est placé dans un étui de cadmium. Le dosimètre est ainsi sensible seulement à la composante épithermique des neutrons incidents et réfléchis; ce qui réduit d'une part la dépendance énergétique et d'autre part l'influence de la position du dosimètre par rapport au corps humain. Des mesures effectuées sur fantôme auprès du réacteur source SILENE ont montré que le rapport : (mesure PTL 716 - mesure PTL 717)/ (mesure PTL 710 - mesure PTL 717) variait entre 4 et 9 suivant la dureté des spectres.

Ces résultats étant très prometteurs, l'étude sera poursuivie en utilisant la variation de ce rapport pour déterminer les caractéristiques énergétiques du spectre.

b) Les détecteurs à traces -

On s'est orienté vers la détection de traces dans le nitrate de cellulose. On a d'abord utilisé une mince couche (100µm) de nitrate de cellulose KODAK CA 80-15 recouverte d'une couche de tétraborate de Li ($Li_2B_4O_7$). Les α des réactions (n,α) sur le bore et le lithium sont enregistrés dans le nitrate de cellulose, insensible au fond γ et comptés sur microscope après attaque chimique. Ce détecteur est pris en sandwich entre deux plaques de plexiglas munies d'écrans de cadmium. On définit ainsi 4 plages de lecture : plexiglas, cadmium "avant", cadmium "arrière", cadmium des 2 côtés. Une irradiation au PTB de Brunswick a montré que les courbes de réponse en fonction de l'énergie étaient sensiblement différentes selon la plage. On peut donc obtenir une indication grossière sur l'énergie des neutrons incidents. (voir figure n° 3 sur laquelle ne sont représentés que les plages plexiglas et cadmium des 2 côtés).

Un nouveau nitrate de cellulose (CN 85) a été utilisé par la suite et un nouveau convertisseur au bore, permettant un gain de sensibilité d'un facteur 4 a été adopté.

Le dosimètre actuel consiste en un assemblage de trois feuilles de CN 85 avec leur convertisseur, séparées par deux écrans de cadmium de 40 mm de diamètre. (voir la figure n° 4).

Bien qu'il soit encore nécessaire de réaliser de nouvelles expériences pour la mise au point, nous considérons que les résultats acquis sont très encourageants.

En conclusion de cette première partie, les résultats obtenus montrent les voies à suivre pour mettre au point un dosimètre (à traces ou thermoluminescent) dont la variation de la réponse en fonction de l'énergie serait auto-corrigée. Les études en cours montreront l'intérêt

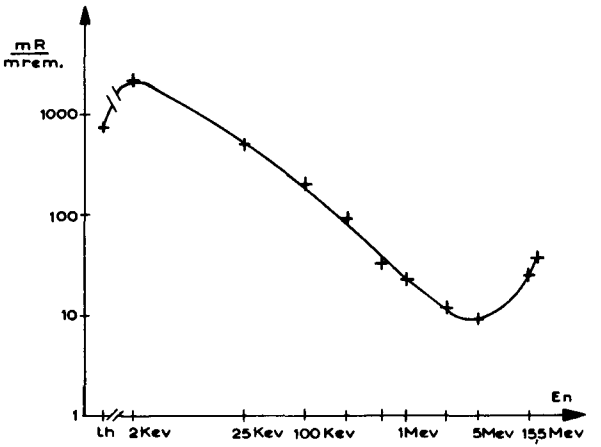

Fig.1 - **Réponse du dosimètre de HARVEY (Fli^N_Fli^7)**

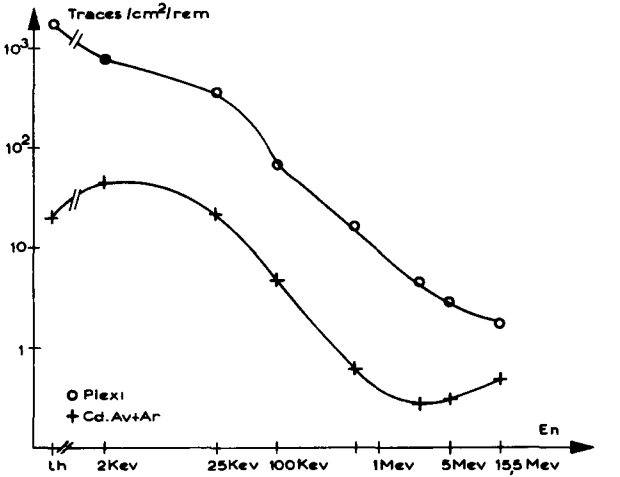

Fig.3 _ **Dosimètre à traces CA 80.15**

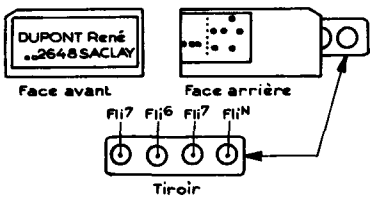

Fig.2 - **Dosimètre thermoluminescent PGP1**

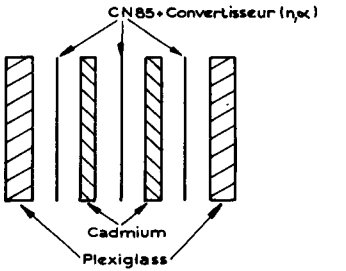

Fig.4 - **Dosimètre à traces au CN85**

respectif des 2 techniques étudiées.

2) Emission exoélectronique thermostimulée -

Dès le départ de l'étude en 1977, on disposait d'un lecteur performant pouvant fonctionner en thermostimulation ou photostimulation. Les premières expériences ont servi à vérifier la "faisabilité" de la mesure des ionisations induites dans le détecteur par les protons issus d'un radiateur hydrogéné. Nous avons utilisé pour cela un détecteur composé d'alumine α (exoémettrice) et de graphite (conducteur) déposés par simple sédimentation, on a obtenu pour une source Pu-Be un rendement $R\frac{n}{\gamma} = 0,4$.

($R\frac{n}{\gamma}$ = réponse aux neutrons/réponse aux γ)

Des essais ont été ensuite effectués avec de l'alumine β comme conducteur ionique; la réponse est accrue : $R\frac{n}{\gamma} = 0,6$ grâce à l'amélioration de collection des exoélectrons.

Pour des raisons pratiques nos efforts ont également porté sur la réalisation de détecteurs de bonne tenue mécanique et de manipulation aisée.

On s'est pour cela orienté vers la pulvérisation des alumines $\alpha + \beta$ dans une flamme de chalumeau à plasma sur un support en acier inoxydable.

On peut réaliser des dépôts d'épaisseur variée et les caractéristiques dosimétriques des échantillons sont bien conservées. Les couches ainsi obtenues sont bien adaptées à la détection des protons de recul, à condition d'éviter qu'une partie de l'alumine α ne soit transformée en alumine γ non exoémettrice lors de la projection à haute température. Des traitements thermiques destinés à reconvertir la phase γ en alumine α sont actuellement à l'étude.

Les résultats sont d'ores et déjà satisfaisants. Un lecteur semi-automatique est en cours de réalisation. L'étude d'un radiateur hydrogéné (stratifié de polyéthylène et d'aluminium mince), permettant d'obtenir, en fonction de l'énergie des neutrons, une réponse voisine de l'équivalent de dose, est largement avancée.

En conclusion de ces travaux, on peut considérer que les principaux obstacles techniques ont été surmontés; la réussite de ce procédé dépend désormais uniquement de la mise au point du radiateur hydrogéné destiné à corriger la variation de réponse en fonction de l'énergie.

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Optimization of flame-sintered Al_2O_3 Exoelectron Dosimetry Materials
6th International Conference on Solid State Dosimetry. Toulouse - France (1-4 April 1980)
Nuclear Instruments and Methods 175 - (1980) 115-116

Contractor of the Commission:	Physikalisch-Technische Bundesanstalt, Braunschweig Abteilung Atomphysik
Contract No.:	108-77-1 PST D
Head of research team:	Prof. Dr. S. Wagner
General subject of Contract:	Improvement of methods for neutron spectrometry

In radiation protection from neutrons which can be considered as penetrating radiation, exposure of the whole body is generally restricted by the limits applying to the "effective dose equivalent" arising in the exposed person. However, the effective dose equivalent cannot be determined by direct measurements, as it is defined as the sum of weighted average dose equivalents in various organs or tissues. In consideration of this problem, ICRP has specified secondary limits for the "dose equivalent index". But this quantity, as defined by ICRU, is not additive in respect to different radiation components distributed in energy and direction, and hence not even an ideal instrument could be designed which would indicate the dose equivalent index unambiguously. In order to cope with this situation, operational quantities have been introduced for the purpose of practical measurements, dose equivalent ceiling being one used for area monitoring of neutrons. However, no primary standards for the realiziation of the unit of this quantity exist, as quality factors are involved in the definition of all dose equivalent quantities, which are a function of the linear energy transfer at the point of interest in the irradiated body. A remedy for this difficulty is to relate quantities characterizing the radiation field in the absence of the irradiated body to the operational dose equivalent quantity by means of calculations. Conversion factors are available as a result of such calculations, relating neutron fluence to dose equivalent for monoenergetic incident neutrons. For the practical calibration of survey instruments, however, radioactive neutron sources are frequently used, emitting neutrons of a broad energy spectrum. In order to evaluate the conversion factor to be applied with such sources, this spectrum has to be known with sufficient accuracy. This contract aims at improving the methods used for neutron spectrum measurement and testing them with neutron sources suitable for calibrating radiation protection instruments.

Title of project:	Development of a ^{3}He spectrometer for the measurement of the fast neutron energy distribution
Head of project and scientific staff:	Dipl.-Phys. H. Kluge

The aim of the work was to develop a ^{3}He spectrometer for the determination of spectral distributions of fast neutrons. Knowledge of the neutron energy spectrum is of importance when radionuclide sources emitting neutrons with broad energy spectrum are used for calibrating radiation protection instruments, which must usually be calibrated in units of the general quantity dose equivalent.

As to date no practical and accurate method exists for the direct and absolute determination of any dose equivalent quantity for neutrons, it is necessary to measure radiation field quantities (neutron fluence, its spectral and angular distribution) and to evaluate dose equivalent quantities by means of fluence-to-dose equivalent conversion factors. The energy range below 1 MeV is of special interest because of the strong dependence of the conversion factors on energy and as in practice, neutrons of this energy range may contribute considerably to the total dose equivalent.

The spectrometer consists of two surface barrier detectors (active area 450 mm^2, distance 10.3 mm), the space between them filled with ^{3}He and operated as a proportional counter /1/ (cf. Fig. 1). If the reaction ^{3}He(n,p)^{3}H is induced by a neutron in the sensitive volume and the proton and the triton are emitted so that their energy losses ΔE are measured by the proportional counter and their residual energies E_1' and E_2' by the semiconductor detectors, then the neutron energy E_n can be calculated from

$$E_n = E_1' + E_2' + \Delta E - Q, \quad Q = 764 \text{ keV}.$$

Metallic sealings were exclusively used in the construction of the spectrometer in order to prevent loss of Helium-3. After scrupulous surface treatment of all inner parts of the spectrometer and a thorough purification of the He gas, stable performance of the proportional counter over a period of several weeks was attained, which is a prerequisite for the investigation of weak neutron sources.

Total energy resolution of the spectrometer depends mainly on the properties of the proportional counter. Several cathodes of various shapes were

investigated finally leading to the design of a rectangular cathode with plane walls of quadratic cross-section. A weak ^{241}Am α-particle source was mounted in the middle of a side-wall for calibration purposes. As the gas amplification process can be stabilized and the drift velocities of electrons increased by an admixture of polyatomic gas, the influence of carbon dioxide and methane was investigated. The best results were obtained with CO_2 at a relative partial pressure of about 2.3 % when the total pressure was 1.3 bar.

Measurements with monoenergetic neutrons in the energy range from 250 to 5000 keV confirmed the correctness of the calibration with the built-in α-particle source and showed that energy resolution of the spectrometer is almost independent of neutron energy with a line width of about 70 keV (full width at half maximum).

Data acquisition was performed by means of a multiparameter analyzing system /2/. A PDP 11 computer was used for the on-line analysis of the signals from the spectrometer. For coincident signals, a two-dimensional matrix of the energy loss ΔE of particles in the proportional counter from the various possible reactions versus total energy $E_1'+E_2'+\Delta E$ is being set up. Parameter-dependent "table cut" conditions /2/ TCT1 and TCT2 are defined separating signals due to photons (below TCT1) and signals mainly from Si(n,α) reactions (above TCT2) from those produced by the reaction $^3He(n,p)^3H$ and Si(n,p) reactions in the semiconductor detectors (cf. Fig.2). Projections on the total energy axis are separately accumulated on-line for each region with a four times better resolution. In this way, an almost complete separation of photon signals from neutron signals can be achieved for exposure rates of about 0.5 R/h, which was the highest value occurring in the measurements up to now. The remaining contribution to the background pulses which is due to Si(n,p) reactions was measured by filling the spectrometer with ^4He gas, leaving all other conditions unchanged.

A similar data acquisition system is implemented on a Siemens R30 computer.

The efficiency of the spectrometer was measured by means of neutrons from a ^{252}Cf spontaneous fission source of known source strength by comparing the measured pulse height distribution with the theoretical spectrum, which can be described by a Maxwell distribution with a spectrum parameter of T = 1.42 MeV in the energy range from 1 keV to 10 MeV /3/. In the range

from 250 keV to 8 MeV, the theoretical spectrum does not deviate from the Maxwellian by more than ± 5 % /4/, but larger deviations of up to 10 % may arise at its ends /3/. This calibration procedure was checked by measurements with monoenergetic neutrons of known fluence in the energy range from 250 keV to 5 MeV, showing no significant discrepancies when taking into account the uncertainties of the measurements of about 5 % (standard deviation). For higher energies, the uncertainty of the measured efficiency increases due to the decreasing amplitude in the neutron energy spectrum of the ^{252}Cf fission.

Two measurements of the neutron energy spectrum of a ^{241}Am-Be(α,n) source (source strength $3 \cdot 10^6$ s^{-1}, diameter 25.2 mm, height 25.2 mm) were performed, the neutrons being unaffected by scattering and attenuation. This source is used at the PTB to produce a standard irradiation field for the calibration of radiation protection instruments. Fig. 3 shows the result of the measurements /5/ compared with a theoretical spectrum calculated by Geiger and van der Zwan /6/. Unfolding of the measured spectrum was not necessary because of the high resolution of the spectrometer /5/. The lower energy limit for these measurements was about 100 keV.

Summary: A ^3He spectrometer has been developed for the determination of the fast neutron energy distribution in the range of about 100 keV to 12 MeV in mixed radiation fields. This range is especially important for the neutrons emitted from radionuclide sources with broad energy spectra which are frequently used in the calibration of radiation protection instruments.

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/3/ Blinov, M.V.: Neutron Energy Spectra of Spontaneous Fission Sources. Proc. IAEA Consultants' Meeting on Neutron Source Properties, IAEA Vienna 1980, INDC(NDS)-114/GT, 79-106.

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/5/ Kluge, H.; Weise, K.; Klein, H.: To be published.

/6/ Geiger, K.W.; Van der Zwan, L.: Radioactive Neutron Source Spectra from ^9Be(α,n) Cross Section Data. Nucl.Inst.Meth. 131 (1975), 315-321.

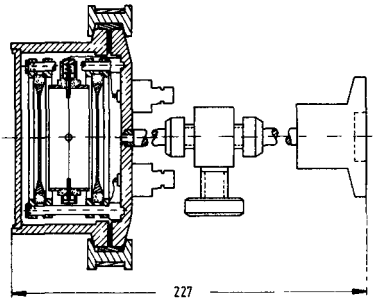

Figure 1: Buildup of the spectrometer

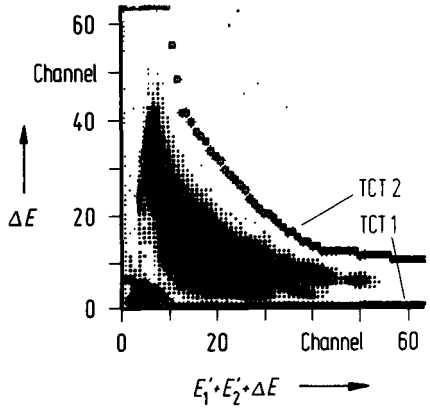

Figure 2: On-line analysis with parameter-dependent cut conditions (TCT1, TCT2) of the two-parameter spectrum of energy loss ΔE versus total energy $E_1'+E_2'+\Delta E$

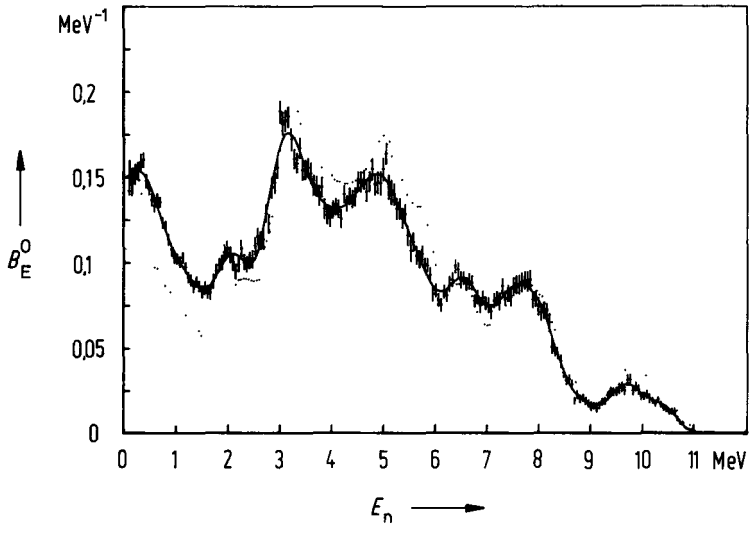

Figure 3: Measured neutron energy spectrum of a ^{241}Am-Be(α,n) source. Full line: Fourier-fit with 30 functions fulfilling χ^2-test. Dotted: Theoretical spectrum calculated by Geiger and van der Zwan /6/. E_n neutron energy, B_E^o normalized spectral source strength.

Contractor :	National Radiological Protection Board, Harwell, Didcot, Oxon OX11 ORQ
Contract No. :	111-79-1 PST UK
Head of Research team :	D.F. White
General subject of Contract :	The development of a rationalised approach to the measurement of external beta and gamma radiation for radiation protection purposes.

Title of Project No. 1 :	The development of a rationalised approach to the measurement of external beta and gamma radiation for radiation protection purposes.

Head of Project and scientific staff: Mr. T.M. Francis, Mr. D.A.J. Morris

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The objective of this project was to establish experimentally the relationships between the 'free-air' radiation quantities hitherto used in standards laboratories and radiation protection instruments, and the dose equivalent and dose equivalent index quantities defined in ICRU Report 25 and ICRP Publication 26.

The density and attenuation characteristics of a range of materials were studied as a result of which a composition coded MS20 (White, 1974) was chosen for the construction of a 30 cm diameter tissue equivalent sphere corresponding to that specified by the ICRU in the definition of dose equivalent index. Considerable difficulties were initially experienced due to frothing caused by the exothermic nature of the setting agent and the fact that the mechanical characteristics of the final product depended critically on the particular batch of magnesium oxide used as an ingredient. The sphere was eventually constructed successfully by adding the material in thin layers to the casting vessel and allowing each layer to set in vacuo. It was constructed in three parts consisting of two hemispheres and a central disc 1.5 cm in thickness. Narrow slots, each of which would accommodate three thermoluminescent dosemeters, were machined in the central disc over a range of depths from 0.3 to 15 cm and along seven radii spaced 30° apart. Several TLD materials were evaluated and LiF-teflon TLD discs of 0.2 or 0.4 mm thickness were chosen. The slots can accommodate a total of 90 of these discs. In order to obtain the required accuracy and reproducibility each dosemeter was

calibrated both before and after exposure in the sphere.

The dose distributions within the sphere were determined using substantially mono-energetic x-rays of energy 16.6 keV, 25.2 keV, 30.9 keV, 48.8 keV, 74.1 keV and 97 keV obtained from a fluorescent x-ray generator, mean energies of 109 keV obtained from a filtered x-ray generator, and 661.6 keV and 1.25 MeV obtained from ^{137}Cs and ^{60}Co sources respectively. Measurements with x-rays of energies between 120-250 keV are in hand but are not yet completed. The free-air dose rates at the position of the centre of the sphere were determined in each case by means of ionisation chambers, the calibration of which was directly traceable to the national standards. In general, the radiation qualities conformed to the reference radiation qualities specified by the ISO. As examples of the results obtained the central axis dose equivalent distributions versus depth within the sphere for energies of 25.2 keV, 48.8 keV, 74.1 keV, 109 keV and 661.6 keV are shown in Figure 1. These clearly show the substantial contribution from scattered radiation within the sphere in the intermediate energy range. The data can be used to obtain a variety of dose equivalent quantities related to the ICRU sphere. The deep dose equivalent index, which is numerically equal to the dose equivalent at a depth of 1 cm in the energy region studied is shown in Figure 2 and agrees reasonably well with theoretical Monte-Carlo calculations (Dimbylow and Francis 1979).

The depth dose distributions in tissue, per unit absorbed dose in air, have been measured for the following beta emitting nuclides, ^{147}Pm, ^{204}Tl, $^{90}Sr/^{90}Y$ and $^{106}Ru/^{106}Rh$, using the tissue equivalent material MS20 and the extrapolation chamber. Particular emphasis was placed on the depths of 0.3 cm (eye lens) and 0.007 cm (skin). The variation of the ratio of the doses at these two depths with the E_{max} of the beta radiation source is shown in Figure 2. It may be noted that for all values of this ratio which exceed 0.3, it is possible for the present ICRP dose equivalent limit to the lens of the eye (150 mSv) to be exceeded while the dose equivalent to the skin remains below the current ICRP limit of 500 mSv.

The beta depth dose distributions were measured over a range of angles, 0°-60°, between the source and the normal to the tissue surface and Table 1 gives the relative doses, normalised to unity at the surface, at two depths of interest for the $^{90}Sr/^{90}Y$ source. The observation that

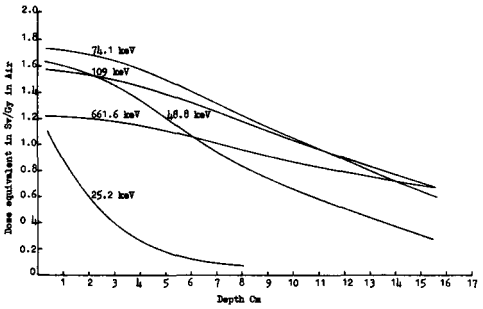

Figure 1. Distribution of dose equivalent with depth along the central axis of the sphere for a range of photon energies.

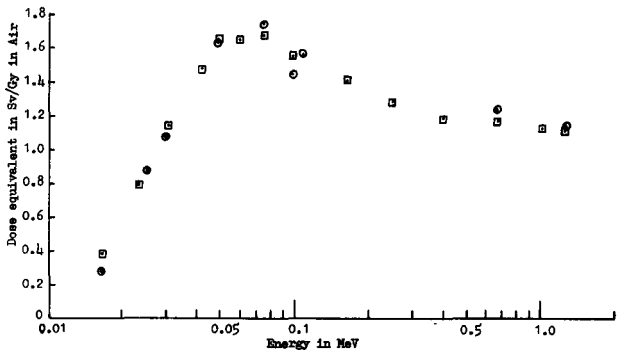

Figure 2. Dose at 1 cm depth in sphere (= deep dose equivalent index) versus photon energy. ⊙ Experimental, ◘ Theoretical.

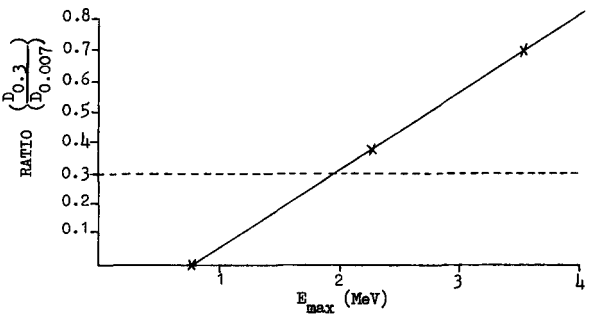

Figure 3. Ratio of doses at depths of 0.3 cm and 0.007 cm as a function of the E_{max} of beta-emitters.

Depth in Tissue (cm)	Source Orientation		
	0°	30°	60°
Surface	1.00	1.00	1.00
0.007	1.07	1.11	1.04
0.3	0.41	0.48	0.48

Table 1. Variation of dose at various depths in tissue with source direction for $^{90}Sr/^{90}Y$.

the dose, even at a depth of 0.3 cm, does not depend markedly on the direction of the source indicates the diffuse nature of the incident beta particles.

The results described in this work provide data for the estimation of a variety of dose equivalent quantities in the ICRU sphere and the determination of the conversion factors between these and the free-air quantities currently used for calibration purposes. Such quantitative data should be of assistance in deciding on the most appropriate quantity for external beta and gamma radiation. A report containing all the results and details of the experimental procedures used is in preparation.

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III. 2.

RADIOAKTIVE KONTAMINATION DER UMWELT

RADIOACTIVE CONTAMINATION OF THE ENVIRONMENT

CONTAMINATION RADIOACTIVE DU MILIEU

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Tätigkeitsbericht beschrieben : *

Further research work on these subjects will also be described in the following progress reports : *

D'autres travaux sur ce thème de recherche sont également décrits dans les rapports suivants : *

272-BIO F	INRA, Dijon (Dalebroux)
273-BIO F	Univ. Toulouse (Delpoux)
274-BIO B	CEN, Mol (Léonard)
179-BIO UK	NRPB, Harwell (Dennis/Smith)
207-BIO D	GSF, Neuherberg (Kriegel)
233-BIO B	CEN, Mol (Vanderborght)
099-PSA F	CEA, CEN, Fontenay-aux-Roses (Uzzan)

* Siehe auch Punkt IV,
See also section IV,
Voir aussi point IV,

Contraente della Commissione : Comitato Nazionale per l'Energia Nucleare (C. N. E. N.)

Laboratorio per lo Studio dell'Ambiente Marino
I 19030-Fiascherino (La Spezia).

N. del contratto : 172-76-1-BIO I

Capo dei gruppi di ricerca : Arrigo CIGNA

Tema generale del contratto : "Investigation for the evaluation of the impact of nuclear plants discharges in the marine environment, under the environmental and health protection standpoints".

Titole del progetto n. 1 : "Description and classification of typical marine coastal ecosystems"

Capo progetto e collaboratori scientifici : Dr. Roberto Boniforti, Dr. Aldo Brondi, Dr. Giuseppe Buffoni, Prof. Arrigo Cigna, Dr. Alessandro Delle Site, Dr. Alfredo Esposito, Dr. Ornella Ferretti, Dr. Antonio Zattera, Dr. Giancarlo Zuccaro Labellarte, Dr. Giovanni Zurlini.

By taking into account the suggestions of the Management Committee, the program for 1980 as reported in the 1976-1980 research program with CEC has been modified into the version included in the research contract 1980-1984. In particular the different topics have been as far integrated as possible in order to improve the output of the whole working group.

A geomorphological classification has been used to select the most representative sampling points in the zones investigated, namely, Ligurian Sea in front of La Spezia, Tyrrhenian Sea in front of Latina's nuclear power plant, and Tyrrhenian Sea in front of Garigliano's nuclear power plant.

As far as the characterization of Italian coasts is concerned, the following results have been obtained. The coasts ($\sim$ 7000 km) can be classified in cliffed (55 %) and low ones (45 %). The latter cor-

respond to delta (20 %) alluvial (10 %) and dune plains (5 %), as well as to emerged marine terraces (10 %). Only 25 % of the coastal plains are less than some hundreds of meters wide. An important part of the coasts is subjected to erosional processes. About 180 samples collected in rivers and on beaches have been analysed. The resulting mineralogical composition (quartz, feldspars, carbonatic and clay minerals), together with the knowledge of the distribution pattern may provide basic information on the behaviour of radionuclides in the different types of coastal sedimentary environments of Italy.

The effects of wind on deep waters of the Ligurian Sea and the interactions of deep waters with coastal currents have been studied by a barotropic linearized analytical model.

The distribution pattern of the water from Magra River is also confirmed by the results of the analysis carried out on sediments samples collected at the most peculiar sampling points selected according to the geomorphological scheme. In fact, the solid transport of the river is by far the predominant factor controlling the composition of the sea sediments.

The analysis of some lacustrine sediments collected from the Massaciuccoli lake containing higher amounts of C org and C inorg than marine sediments, showed also a concomitant higher concentration of Co, Cu and Mn than in the marine sediments.

The results of some measurements of ^{137}Cs concentration in undisturbed core sediments samples show higher values in areas with higher sedimentation rate (e.g., an average value 450 pCi/Kg DW inside the Gulf vs. 150 pCi/Kg DW in front the Magra river mouth). Such higher values are also found in comparable situations from the point of view of sedimentation of small size particles (i.e. Gaeta Gulf 550 pCi/Kg DW).

On the other hand in the open sea sediments the values are quite similar to those obtained along other stations of the Italian Coast. Also the ^{137}Cs concentration in fishes and mussels show no difference with reference to other sampling points along the Italian coasts.

The relationship between the distribution of artificial radioactivity levels and local morphological and sedimentological characteristics was also established in the Tyrrhenian sea in front of the Latina nuclear power plant. In particular, the distribution of fall-out-borne ^{137}Cs was inversely related to the grain-size of sediments.

A general trend was observed by which, at higher depths, smaller sedimentary particles and higher radioactivity levels of ^{137}Cs

were present. Deviations from this expected behaviour were due to the presence of particular morphological characteristics or phanerogams meadows which enables the sedimentation processes of small particles to take place.

^{137}Cs showed maximum activity in sediments collected from phanerogams meadows. These sediments are characterized by a high organic content and a large amount of small-size particles. The vertical profiles of ^{137}Cs show a maximum activity (300 pCi/Kg DW at 8-10 cm depth). The distribution pattern of 239, 240 Pu is similar, but maximum activity (40-50 pCi/Kg DW) occurs at 15-20 cm depth.

The patterns observed are probably due to the sedimentation processes near the coast involving small particles of land origin, which appear to scavenge ^{137}Cs and 239, 240 Pu at different rates.

For sediment cores collected from an area with great bottom variability (Foce Verde/Latina), the concentration of ^{137}Cs is inversely related to the grain-size of sediments and not to the mineralogical composition. Sediments collected from zones subjected to a limited hydrodynamism, where mainly particles of small and very small size are present, show the highest levels of ^{137}Cs, independently on the mineralogical composition.

Obviously, it cannot be excluded the intervention of other factors in determining the observed association between small particles and artificial radioactivity levels (e.g. the organic contents of sediments, chemical-physical processes, etc.

Preliminary hydrological measurements have been made at the Garigliano site, (facing a nuclear power plant in operation since 1963) with the aim of planning the study to be made in 1981. This study, based on hydrological parameters and current measurements, will be aimed at investigating the dispersion of the effluents released from the nuclear plant. The results of the dispersion model will be tested by using the results of radioactivity measurements made in that zone.

A radioecological survey in the same marine environment has been started in cooperation with other CNEN and CNR laboratories. The preliminary results show that ^{60}Co is present in many matrices of that area (e.g. 150-1600 pCi/Kg DW sediments; 40 pCi/Kg FW fish Mugil cephalus; 10 pCi/Kg FW mollusc Mytilus galloprovincialis).

The distribution pattern of ^{60}Co released from the nuclear power plant gives a unique tool for further research on both the pathways of this radionuclide in the marine environment and application of

models.

A twice per year collection of abiotic and biotic samples from 5 stations has shown that the only artificial radionuclide detectable is 137 Cs and that it is homogeneously distributed along the Italian coasts (e.g. 5-10 pCi/Kg FW fish Engraulis encrasicholus, 100-200 pCi/Kg DW sediments).

The final analysis of the data collected at the La Maddalena Archipelago during past years showed that currents in the Archipelago are strictly dependent of meteorological conditions. Based on this dependence, a numerical model for the circulation of water masses was developed. The boundary conditions of the model were established by simulating the circulation pattern in a wider zone including the Bocche di Bonifacio. Expected and experimental data agree.

This environmental research has been started during 1975; field investigations were carried on also in the successive years. The studies in physical oceanography have been performed in cooperation with the Oceanographic Station of S. Terenzo (La Spezia) (National Council of Research). During 1976 four cruises have been made using the oceanographic vessels Odalisca (CNEN) and Marsili (CNR), as well as vessels of the Navy and rented boats in the following years.

The aim of the environmental survey carried out at La Maddalena is the evaluation of the limit capacity of this site from the health and, more generally, radioecological viewpoints under conditions of possible discharges also used for predictions of the behaviour of radionuclides in the sea under accident conditions.

Samples have been collected on the basis of a monthly frequency in four stations and of semi-annual frequency in ten stations. The following radionuclides have been determined: Ce 144, Ce 141, Ru 103, Rh 106, Cs 137, Zr 95, Nb 95, (fission products), Mn 54 and Co 60 (activation products, detected in extremely low concentration in Pinna nobilis). The contamination from fission products is related to atmospheric fallout. The maximum level of radioactivity measured during 1977 is $\sim$ 10 pCi/g (DW) of Ce 144 and occurred in Posidonia sp.

Microbial activity, concentration of sulfate reducing bacteria, and organic matter content have been determined due to the role that the interplay of these factors seems to have on both the immobilization and mobilization of elements in/from sediments. The maximum respiratory capacity of bacteria, the concentration of sulfate-reducing bacteria, and the content of carbon (organic and inorganic)

and total nitrogen have been determined.

Preliminary results indicate that the surface layer of a sand sediments and mud collected from the La Spezia Gulf have an oxygen consumption of about 0.2 and 1.1 micromoles of O_2 per gram (DW) respectively.

Titole del progetto n. 2 : "Laboratory studies on the behaviour of the long-lived radionuclides in the marine environment".

Capo progetto e collaboratori scientifici : Prof. Pietro Scoppa, Prof. Arrigo Cigna, Dr. Ernst Schulte, Dr. Antonio Zattera.

The program proposed for the last year of the contract 1976-80 was replaced with a new one, based on the research priorities underlined by the Management Committee and the Radiation Protection Program 1980-84.

Research on mechanisms determining the behaviour of long-lived radionuclides are certainly relevant in relation to the evaluation of collective dose and collective dose committment.

Activities on technetium began according to the proposal for collaboration with Dr. Fowler (IAEA, Monaco), tendered by Dr. Schulte to the Management Committee (3rd Meeting, Jan. 1980). This collaboration made available to us the isotope Tc-95m, a gamma-emitting nuclide particularly suitable for experiments on accumulation, distribution and release of technetium by marine organisms. The use of a gamma-emitting isotope was a great advantage under several points of view (low concentrations, existing instrumental facilities, whole-body counting, etc.). The beta-emitting isotope Tc-99 was also used in several experiments, where relatively high concentrations of technetium proved to be necessary.

Preliminary experiments showed the high stability of the pertechnetate in aqueous solution, over a wide range of pH and dissolved oxygen concentration. That anion can be considered as the prevalent form of technetium in natural waters. Therefore, it was decided to use pertechnetate in the first phase of our esperiments with marine organisms.

Possible interactions of pertechnetate with naturally occuring polymers and biological macromolecules were studied using dialysis or diafiltration (chemical interactions), filtration or centrifugation (adsorption phenomena). Alginates, chitin, chitosan, humic substances, albumin, nuclei acids don't bind pertechnetate under the experimental conditions used in our investigation.

The UV-absorption characteristics of pertechnetate were determined using Tc-99, at concentrations of ~ 0.1 mM. Working at high resolution (0.1 nm) could be resolved into two peaks: the first maximum at 244 and 248 nm, the second at 287 and 292 nm. Further details on the UV-spectrum of pertechnetate were obtained by scanning a first derivative spectrum: the presence of many maxima and minima showed that the original spectrum contains a relevant number of inflection points.

This knowledge was utilized to have preliminary information on the spectral modifications produced by reducing agents, such as hydrochloric acid, stannous chloride, hydroxylamine, hydrazine and cysteine. High concentrations of hydrochloric acid were required to reduce pertechnetate. Stannous chloride in acidic solution proved to be very effective, while the sulfates of hydroxylamine and hydrazine did not produced modifications of the UV-spectrum of pertechnetate. Of particular interest the reaction of pertechnetate with cysteine: it is a slow process leading to the formation of a product absorbing in the visible region (420 nm).

A spectrophotometric study of this process showed that it is first order with respect to pertechnetate, while a large excess of cysteine is required to have measurable rates of A at 420 nm. At present it is too early to suggest possible mechanisms involved in the sequence of reactions occuring when pertechnetate is in the presence of much higher concentrations of cysteine. However, it would be interesting to investigate a possible biological role of this process.

Short-term experiments directed to evaluate possible chemical effects of pertechnetate on phytoplanktonic organisms were performed. The range of concentration tested was extended up to 10 mg Tc/liter. Neither toxic behaviour nor modifications of growth rate were observed in cultures of Phaeodactylum tricornutum and Dunaliella sp.

From these and other experiments with Tc-95 m (concentration in the cultures: ~ 1 pg/l), it could be shown that the phytoplanktonic species taken into consideration don't accumulate technetium to any appreciable extent.

Accumulation of technetium from seawater by the shrimp Palaemon elegans was studied at two acclimation temperatures (10° and 20° C). The concentration factor at equilibrium was in both cases ~8, while the turnover was almost doubled at the higher acclimation temperature. The radioactivity was found mainly in the viscera: at the end of the experiment the concentration factor for hepatopancreas had a mean value of 150. Accumulation of technetium from contamined food (Artemia salina) showed that after two weeks there is little accumulation: approximately 20 % of the total activity ingested is still present in the body. The transfer of radioactivity from stomach to hepatopancreas is very rapid: ~85 % of the daily dose is already in the hepatopancreas and ~10 % excreted with faeces, 16 h after a contaminated meal. Considering that the radioactivity accumulated at the end of the experiment represents ~20 % of that ingested with food, it arises that the technetium present in hepatopancreas should have a relatively short biological half-life. This observation had been confirmed by an experiment in which Palaemon were fed with contaminated Artemia for two weeks, then sacrified at several time intervals after last meal, dissected and the organs counted for their radioactivity. The biological half-life of technetium present in the hepatopancreas was ~120 h. The radioactivity of the other organs was toolow to permit calculations of biological half-lives. The loss of technetium accumulated from seawater or from contaminated food was also studied in whole shrimp. Although the radioactivity in both cases was almost entirely localized in the hepatopancreas, the kinetics of elimination were somewhat different: biphasic when accumulation was from water, monophasic when accumulation was from contaminated Artemia. Most of the technetium accumulated from water (~70 %) was eliminated at a fast rate, the remaining fraction being excreted at a slower tal amount of technetium accumulated from food. Although the presence of the slow phase requires confirmation by further experiments with higher concentrations of technetium, these observations suggest that in the hepatopancreas of Palaemon elegans the technetium could be transformed to less mobile chemical forms. The metabolic conversion does not occur when the isotope had been accumulated from food, probably because the technetium had been previously metabolized by Artemia.

Preliminary experiments on the accumulation of technetium from seawater were performed with other marine organisms. The results can be summarized as follows:

Artemia salina - conc. fact. at equilibrium = ~3
Scyllarus sp. - conc. fact. after 8 days = ~4
Palinurus vulgaris - conc. fact. after 8 days = ~5

Enteromorpha intestinalis - conc. fact. after 10 days = ~0.6
Nereis sp. - conc. fact. after 7 days = ~300
Marphysa sp. - conc. fact. after 2 days = ~ 350

In despite of the short duration of these experiments, it appears clearly that the species of crustaceans taken into consideration and the only seaweed tested don't concentrate technetium to any remarkable extent, in constrast to the polychaetes in which accumulation occurs rapidly and high concentration factors at equilibrium should be expected.

As far as the old program is concerned, experiments on the accumulation of Cr 51 (as chromate) by river and marine sediments marine bacteria (probably belonging to the genus Pseudomonas), and sediment-supported bacteria has been made for times ranging from 5 to 99 h. The distribution coefficients of sediments (DW) are in the order of 40, while the concentration factor for bacteria (FW) are in the order 12,200 (FW).

Experiments on the release of Cr 51 from laboratory-contaminated sediments on leaching with different solutions indicate that the major percentage of Cr 51 is bound to carbonates and Fe hydroxide while the amount of Cr 51 bound to Mn oxides is negligible under our experimental conditions.

The influence of water temperature on the release of chromium by Dicentrarchus labrax was studied. The release of chromium 51, administered by stomach tube as a solution of sodium chromate, has been followed in two groups o fishes acclimated at 10 and 20°C. Two phases of loss could be easily found: a fast phase, accounting for ~80 % of initial retention, and a slow phase accounting for the remaining 20 %. The relative contribution of the two phases was not significantly modified by the temperature, while the half-life of the short lived component was strongly affected: 11.1 ± 0.9 h at 10°C and 4.1 ± 0.2 h at 20°C. In the second phase the biological half-life (~150 h) was not influenced by the temperature, suggesting that chromium was incorporated in a body compartment with low metabolic activity.

The effects of temperature on phytoplankton were studied through an investigation "in situ" on natural populations of phytoplankton in proximity of the discharge canal of an electricity generating plant. Survival was slightly affected only during the summer period. The study of long-term effects on grouth and survival of populations cultured under laboratory conditions has shown that Dunaliella sp. (Chlorophyceae) resistes at temperature as high as 30°C, while the

thermal resistance (t_{max}) of the other species taken into consideration (all diatoms) is in the range of 25-30°C. Median lethal temperatures for different algal species have been obtained from laboratory experiments simulating the thermal shock caused by passage through a steam condenser.

The effects of thermal shock on Leander squilla were investigated. Median resistance time before loss of equilibrium and median survival time after thermal shock have been determined for individuals acclimated to different temperatures ranging from 5 to 29°C. Experimental data wre submitted to probit analyses to obtain slope functions and 95 % confidence limits. Regression lines between effective times and shock temperature have been calculated for each acclimation temperature. This procedure allowed the definition of a zone of resistance (time- dependent survival), a zone of tolerance (indefinite survival) and a zone of instantaneous death. The upper incipient lethal temperature i.e. the boundary between the zone of tollerance and the zone of resistance, increases when acclimation temperature is raised from 5 to 25°C. For acclimation temperatures of 25°C or higher, the upper incipient lethal temperature remains constant between 34 and 35°C. Therefore, the value of 35°C can be taken as the ultimate upper incipient lethal temperature.

Experiments on the effects of water temperature on the growth of marine algae have been performed, taking as reference level 18°C. At 25°C the growth of all species examined is not modified. The following temperatures for growth inhibition have been observed: 27°C for Bacteriastrum delicatulum and Leptocylindrus danicus, 30°C for Nitzschia closterium, 35°C for Skeletonema costatum, Glenodinium sp. 1, Nitzschia longissima. At this last temperature, the growth of Gyrosigma fasciola, Chaetoceros affinis Dunaliella sp. and Tetraselmis suecica is not influenced.

In addition, short-and long-term effects of increases in temperature have been measured for artificial populations cultured under laboratory conditions. The results show that Chlorophyceae are more eurythermic than other groups (e.g., Diatomeae, Coccolithophorideae and Peridineae). Short-term effects (thermal shock) on Platymonas suecica show an LT-50 of 28°C under conditions simulating the situation existing in the condenser of a heat exchanger.

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Contractor: Ministry of Agriculture, Fisheries and Food
Contract No.: 219-76-1 B10 UK
Head of Research Team: Dr N T Mitchell
General Subject of Contract: Marine distribution and behaviour of transuranic and fission product radionuclides.

Title of Project 1 : Transuranic radionuclides
Head of Project: Dr R J Pentreath
Other Scientific Staff: J W Talbot, B R Harvey, M B Lovett

The principal objective has been to further our understanding of the environmental behaviour of transuranic nuclides, with particular reference to controlled waste disposal into coastal waters. Initially the programme concentrated on improving the analytical methodology, particularly for the determination of curium, and subsequently on the development of a method for neptunium. Although a final document on the methodologies used at FRL has not yet been prepared, two issues of importance have been reported on: the possibility of supported ^{242}Cm in the Windscale area[1], and the demonstration of two sets of oxidation states of plutonium in Irish Sea water[2].

A number of cruises have been made in all coastal waters of the UK in order to measure concentrations of Pu, Am and Cm in both filtrate and particulate water samples. These data are being evaluated in relation to their concentrations relative to ^{137}Cs, and in relation to the chemical speciation of Pu and the known rates of discharge of each nuclide. Some preliminary data have been published[3] which indicate that the association of the three principal transuranium nuclides with suspended materials is in the order of Am Cm Pu, and that the last of these differs from one area to another, probably as a result of changes in oxidation state.

Sediment core samples have also been taken in large number. These samples have been obtained in order to produce an overall 'budget' of the longer-lived nuclides discharged by Windscale, and a full evaluation of the hundreds of analyses being made will take some time to complete. In the process of making this overall assessment, a number of studies have been made in order to identify the mechanisms by which these nuclides become incorporated into sediments, and the possibility of subsequent remobilisation back to the water column. Using the Pu-oxidation technique[4] a number of interstitial water observations have been made in the Windscale area[3,5]. The general conclusions of these studies have been that plutonium is unlikely to be remobilised, once incorporated, due to the lower oxidation states predominating in interstitial waters. A more

general characterisation of the interstitial environment has also been made in relation to the possible effect on oxidation states[6], and the results of laboratory experiments using ^{239}Np as a tracer have been presented[7].

Were it not for the accumulation of transuranium nuclides by biological materials, the impact of discharges to the marine environment would be very small indeed. Studies on the biological availability of these nuclides have therefore concentrated on food species eaten directly by man. Concentrations of Pu and Am, with some additional data on Cm, were followed over a two year period in the Irish Sea[8,9] and the dose to man resulting from alpha emitters in general in a variety of fish species has been evaluated[10]. The consumption of fish is considered to be of minor importance relative to the shellfish pathways[11] and a more thorough examination of concentrations in crustacea has been made. Although the data obtained have not yet been published in detail, some preliminary observations have been presented[11,12]. The occurrence of ^{237}Np in marine biological materials in the Windscale area has also been demonstrated[11].

In order to delineate pathways of accumulation by marine organisms, and to estimate the rates of uptake and loss, controlled laboratory experiments have been made using ^{237}Pu. Detailed studies with fish have demonstrated interesting differences between teleosts and elasmobranchs[13,14] and some of the studies have been extended to a subcellular level[15]. Comparative experiments have also been made with crustacea (lobsters and crabs), and some of the initial results obtained have been presented[11].

Finally, to complete our understanding of alpha-emitting nuclides generally in the marine environment, and to estimate their relative importance as a source of irradiation both to man and to marine organisms, analyses have been made of U, Th (and ^{210}Po) in a variety of organisms[16]. These data are not only intended to put the introduction of man-made alpha-emitting nuclides into the environment into perspective, but will hopefully identify areas where some degree of similarity exists in the behaviour of the actinide elements.

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Title of Project 2 : Fission products
Head of Project: Mr D F Jefferies
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The major part of the programme has concentrated on the measurement of caesium-137 and caesium-134 in British Isles coastal waters and adjacent seas with the main emphasis on the examination of the distribution of the two radionuclides in seawater[1,4,5]. Relationships have been established between concentrations in both seawater and biota and the rates of discharge from the BNFL fuel reprocessing plant, Windscale, Cumbria[1,2,3]. Measures of the inventory of ^{137}Cs in the seawater compartment of various areas of the Irish Sea, based on data obtained from annual cruises of research vessels, have enabled estimates to be made of residence times and steady state concentrations and a comparison made between pre and post 1976.

Based on the change in inventory between cruises and from the regular sampling of seawater by ferry services, estimates have shown that almost all the ^{137}Cs discharged from BNFL Windscale leaves the Irish Sea by way of the North Channel and a sharp change occurred in the flow of water through the Irish Sea in 1976 and continued at least until mid 1979. With an approximate doubling of the flow rate of seawater there was a subsequent reduction in the residence time and steady state concentration of radionuclides in seawater of the Irish Sea. Distributions of ^{137}Cs in seawater have been examined on major cruises of research vessels off the west and north coasts of Scotland in 1978 and 1979, in the North Sea in 1976, 1978, 1979 and 1980 and in the Norwegian Sea in 1980. The data to 1979 demonstrate that almost all the ^{137}Cs leaving the Irish Sea progresses northward around the coast of Scotland to later enter the North Sea through the Orkney-Shetland channel and through the Pentland Firth. This is followed by a southerly movement down the Scottish and English coasts but with easterly movements from about $58^{\circ}\ 30'N$ to $53^{\circ}N$. The data also demonstrate a northerly flow of water out of the North Sea closely confined to the Norwegian coast into the Norwegian Sea. Analyses of data obtained from the North Sea to 1979 have shown an increase in ^{137}Cs concentrations in seawater of the mid North Sea from 1-2 $pCi.l^{-1}$ in May/June 1976 to 5-10 $pCi.l^{-1}$ in May/June 1978. This follows increased discharges from BNFL Windscale in mid 1974 and increased volume flow rates out of the Irish Sea from 1976 onwards. Data from a cruise in Aug/Sept 1979 demonstrated a slight reduction to between 5 and 8 $pCi.l^{-1}$ in seawater of the mid North Sea.

The principal environmental impact in terms of public radiation exposure is seen through two pathways - external through use of shoreline areas and internal

due to consumption of fish and shellfish. Correlations have been derived between dose discharge rate and dose to both critical groups and populations at large[1,3] (UK and other countries both within and outside the Community). By far the more important pathway, especially as far as the Community is concerned, is that due to fish/shellfish consumption. Direct measurements in samples of fish/shellfish, supplemented by values deduced from water distributions and appropriate concentration factors, have been used to derive estimates of collective whole body dose equivalent. Integrated over the whole population of Western Europe these have ranged from 250 man-Sv in 1976, through 160 man-Sv in 1977 to 210 man-Sv in 1978. Data for 1979-80 will be used to derive further estimates when the fish landing statistics from ICES are available. The data have been used to deduce values for the collective dose commitment, normalised to discharge rate, the most recent evaluations being presented in a paper to an IAEA/NEA symposium in 1980[3]. The most important component of the discharges is ^{137}Cs. A value for the collective dose commitment to Western European countries from fish and shellfish caught from the North and Irish Seas and Scottish waters of about 0.45 man-Sv TBq^{-1} is indicated.

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Contractant de la Commission : Laboratoire de Biologie Moléculaire et Physico-Chimique Université de NANTES

N° du Contrat : 254 77 1 BIOF

Chef du (des) groupe (s) de recherche : Professeur J. PIERI

Thème général du contrat : Distribution des radionucléides rejetés par les installations nucléaires dans un écosystème marin.

Titre du projet n° 1 - Distribution des radionucléides rejetés par les installations nucléaires dans un écosystème marin.

Chef du projet et collaborateurs scientifiques :

- Pr. PIERI
- MM. ANCELLIN
- GUEGUENIAT
- SINE
- GANDON

En 1980 les transferts de radioactivité à partir de l'émissaire de rejet de l'Usine de la HAGUE ont été suivis en utilisant les sédiments littoraux comme indicateurs. Ces études ont été complétées; elles ont été concrétisées par une publication : **"Sediments as indicators of artificial radionucleides distribution West of la HAGUE"** présentée au meeting d'ISPRA (24 - 28 mars 1980) organisé par la Commission des Communautés européennes et l'Agence Internationale de l'Energie Atomique. Deux enseignements sont à extraire de ce travail :

1) Il existe un décalage pouvant attendre un an entre l'instant ou un rejet déterminé est effectué à l'émissaire de la HAGUE et l'observation de son effet sur le littoral de la baie du MONT SAINT MICHEL (100 à 150km à l'Ouest de l'émissaire). La figure 1 qui représente l'évolution comparée des rapports 106 Ru/144 Ce dans des sédiments directement affectés par les rejets industriels (échantillons du Cap de la HAGUE et FERMANVILLE-CAP LEVY d'une part, échantillons de la baie du MONT SAINT MICHEL) d'autre part, témoigne de ce phénomène.

2) Les teneurs du 239 Pu à FERMANVILLE-ZONE témoin pour étudier les effets de l'Usine de la HAGUE- atteignent dans les sédiments 100 à 350 pCi/Kg avec un rapport 238 Pu/239 + 240 Pu de 0,30 à 0,40 en 1979. Les résultats obtenus sur la distribution du 239 Pu dans les sédiments de la Manche sont rassemblés dans le tableau 1.

	^{239}Pu	$^{238}Pu/^{239-240}Pu$	$^{106}Ru+^{106}Rh$	$^{106}Ru+^{106}Rh/^{239}Pu$	$^{106}Ru/^{144}Ce$
FERMANVILLE 27/04/78					
a)	68	0,50	0,600	97	5,5
b)	99	0,31	12 500	126	3,4
c)	98	0,23	21 400	218	4,3
FERMANVILLE 12/04/79					
a)	97	0,30	28 000	287	10,7
b)	350	0,37	160 000	457	10,9
FERMANVILLE 27/07/79					
a)	242	0,40	65 000	268	11,8
BAIE DU MONT SAINT MICHEL (GRANVILLE) 24/03/78					
a)	102	0,22	9 800	96	2,03
b)	134	0,25	13 100	98	2,09
c)	41	0,49	16 100	393	2,07
BAIE DU MONT SAINT MICHEL 21/08/79					
a) Cancale	103	0,22	6 000	58	4,1
b) Le Vivier	288	0,09	7 800	27	4,9
BAIE DU MONT SAINT MICHEL 7/09/79 (VIVIER bas estran)					
a)	53	0,35	2 000	40	
BAIE DE SEINE 20/07/79					
Courseulles	78	0,33	11 000	141	10,1
Ouistreham	77	0,34	10 650	138	9,4
Honfleur	71	0,38	6 140	86	8,6
Le Havre	74	0,24	9 000	122	6,3
Seine	68	0,41	7 800	115	5,8

TABLEAU I - Teneurs (pCi/kg sec) en ^{239}Pu, $^{106}Ru+^{106}Rh$ de sédiments littoraux vaseux de la Manche. Evaluation des rapports $^{238}Pu/^{239-240}Pu$; $^{106}Ru+^{106}Rh/^{239-240}Pu$; $^{106}Ru/^{144}Ce$.

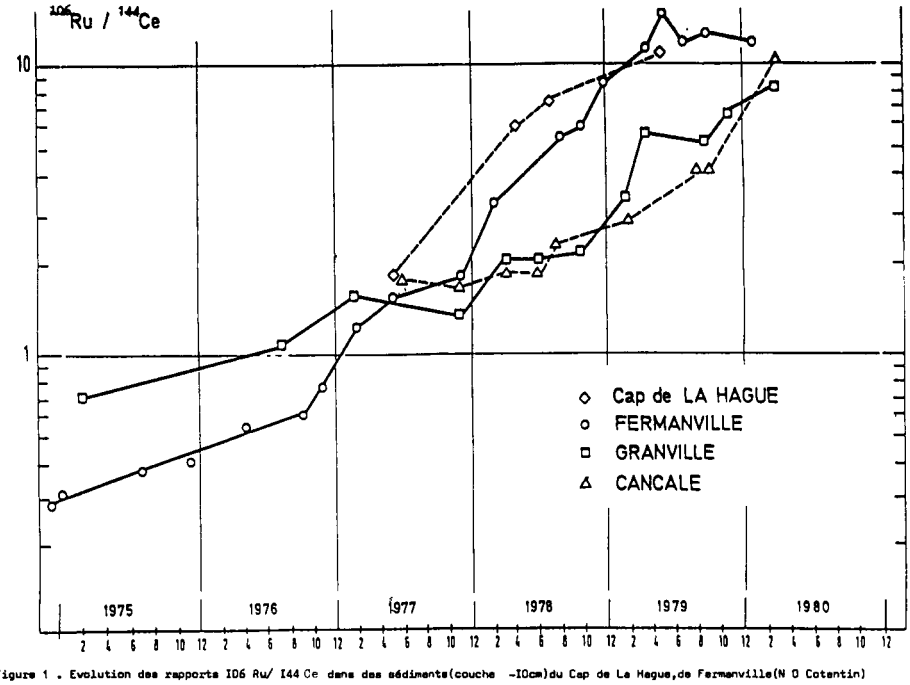

Figure 1 . Evolution des rapports 106 Ru/ 144 Ce dans des sédiments(couche -10cm)du Cap de La Hague,de Fermanville(N O Cotentin) etde la baie du Mont Saint Michel(Granville,Cancale)

En 1980, outre les travaux évoqués ci-dessus, on a porté une attention toute particulière sur la distribution des principaux émetteurs gamma dans les eaux et suspensions de la Manche. Les données recueillies sont transposables à tous les autres radionucléides à condition de faire intervenir le comportement physico-chimique qui joue un rôle essentiel dans la dispersion. Dans le cas présent l'antimoine 125 et le 144 Ce constituent respectivement d'excellents marqueurs des masses d'eau et des particules en suspensions alors que le 106 Ru introduit le terme "source" c'est à dire les caractéristiques physico-chimiques du rejet. L'intérêt présenté par l'antimoine pour suivre les déplacements des masses d'eau est tout à fait remarquable : au moment d'un rejet, avec une charge en matières en suspensions de l'eau de mer de 36,5 mg/l (lors d'une tempête) on a pu constater que la fraction soluble (0,45 m) représentait 99,8 % du total ; d'un autre côté ce radionucléide, à quelques nuances près, ne se fixe que très peu sur les suspensions et les constituants biologiques du milieu. Ainsi en ce qui concerne les sédiments les rapports 106 Ru/125 sb dix fois plus élevés que dans les eaux illustrent du caractère conservatif du 125 Sb au sein de la masse d'eau. Les analyses effectuées au cours de l'année 1980 apportent les principaux enseignements suivants :

1) La radioactivité artificielle observée en Baie de Seine est essentiellement imputable à l'Usine de la HAGUE.

2) Par rapport à la dispersion rapide fréquemment relatée entre le Cap De la HAGUE il existe également une pénétration lente des radionucléides à l'intérieur de la Baie de Seine sous l'effet de courants circulaires analogues à ceux décrits par le modèle de la plaque tournante de GRENOBLE (cf. figure 2).

3) En baie de Seine, pour les raisons indiquées ci-dessus, il se produit un piégeage important du 106 Ru au niveau des énormes stocks de matières en suspensions présents dans cette région (partie reportée sur la figure 3 délimitée par les points 7 8 9, 11 13 16, 12 14 18 19 20, 17, 28 29 30). Il résulte de cet épuisement des masses d'eau du 106 Ru que les rapports 106 Ru/125 Sb qui étaient de 20 au Cap de la HAGUE et dans les effluents de l'usine sont compris entre 5 et 7 en baie de Seine dans la partie chargée en suspensions.

4) Le facteur de dilution d'une zone considérée par rapport à l'émissaire de rejet de la HAGUE peut être exprimé par les rapports entre les activités des divers radionucléides étudiés dans les eaux correspondantes. Ce facteur en baie de Seine est de 7,5 pour le 106 Ru et seulement de 2 pour l'antimoine 125 malgré les 150 km qui séparent cette zone de l'émissaire. On doit constater que les études prévisionnelles qui avançaient un facteur de dilution supérieur à 100, sont loin de refléter les observations effectuées sur le terrain car les études de modélisation ne prennent en considération ni le paramètre temps ni la forme physico-chimique des éléments rejetés.

5) Les données précédentes expliquent certaines anomalies constatées in situ dans la distribution du plutonium et des émetteurs gamma dans divers supports biologiques par rapport à l'émissaire de la HAGUE (faiblesse des facteurs de dilution.

L'essentiel des données précédentes repose sur une campagne océanographique (2) décrite sur la figure 3 et dont les résultats sont reportés dans le Tableau 1.

TABLEAU II - ACTIVITE (pCi/l) DU ^{106}Ru ET ^{125}Sb DANS LES EAUX - B.C.D. ERREURS SUR LE COMPTAGE. B = 5 à 10 %; C = 10-20 %; D = 20-50 %.

	$^{106}Ru(+Rh)$	^{125}Sb	Ru+Rh/Sb		$^{106}Ru(+Rh)$	^{125}Sb	Ru+Rh/Sb
1*	$11,5^{C}$	$1,35^{C}$	8,5	23	$4,6^{D}$	$0,68^{C}$	6,7
2*	$11,6^{B}$	$1,06^{C}$	10,7	24*	$6,8^{B}$	$0,56^{B}$	12,2
2	$12,8^{D}$	$0,86^{C}$	14,0	25	$6,4^{C}$	$0,45^{C}$	14,3
3*	$11,9^{D}$	$1,0^{C}$	11,9	26	$7,3^{C}$	$0,52^{B}$	14,3
3	$9,9^{C}$	$0,77^{C}$	12,9	27*	$4,0^{C}$	$0,58^{C}$	6,9
4	$8,9^{D}$	$0,77^{C}$	11,6	28*	$3,6^{C}$	$0,53^{B}$	6,7
5		$0,94^{C}$		29*	$4,1^{C}$	$0,63^{C}$	6,5
6	8,2	0,63	14,0	29*	$4,7^{C}$	$0,65^{C}$	7,2
7	$2,7^{C}$	$0,4^{C}$	6,8	30*	$3,7^{C}$	$0,60^{C}$	6,1
8*	$3,1^{D}$	$0,79^{C}$	3,9	31*	$3,5^{C}$	$0,40^{C}$	8,8
9*	$3,4^{B}$	$0,52^{C}$	6,5	31	$3,5^{D}$	$0,46^{C}$	7,6
10				32*	$3,5^{D}$	$0,52^{C}$	7,6
11*	$3,7^{C}$	$0,52^{C}$	7,1	32		$0,55^{C}$	
11*	$5,1^{C}$	$0,62^{C}$	8,2	33*	$4,7^{D}$	$0,89^{C}$	5,2
12	$4,6^{C}$	$0,92^{C}$	5,0	33	$5,2^{D}$	$0,55^{C}$	9,5
13	$3,7^{C}$	$0,68^{B}$	5,3	34	$7,9^{C}$	$0,77^{B}$	10,3
14	$4,0^{C}$	$0,61^{C}$	6,5	35	$4,9^{D}$	$0,56^{D}$	8,8
15	$2,2^{D}$	$0,42^{C}$	5,2	36	$11,2^{D}$		
16	$3,3^{D}$	0,65	5,1	37	$16,6^{C}$	$0,47^{D}$	35,3
17*	$3,6^{D}$	$1,0^{B}$	3,6	38	$23,3^{B}$	$1,19^{B}$	18,1
18	$2,9^{D}$	$0,45^{C}$	6,5	39	$17,3^{D}$	$1,22^{C}$	14,2
19*	$4,4^{C}$	$0,67^{B}$	6,6	40	$5,2^{C}$	$0,16^{D}$	32,5
19	$3,0^{D}$	$0,66^{C}$	4,6	41	NM	NM	
20*	$4,6^{C}$	$0,65^{C}$	6,6	42	NM	0,10	
21	$8,1^{C}$	$0,57^{C}$	14,2	43	4,8	NM	< 30
22	$7,8^{C}$	$1,0^{B}$	7,8	44	NM	NM	

X* = analyse MnO_2 - X = analyse $Fe(OH)_3$

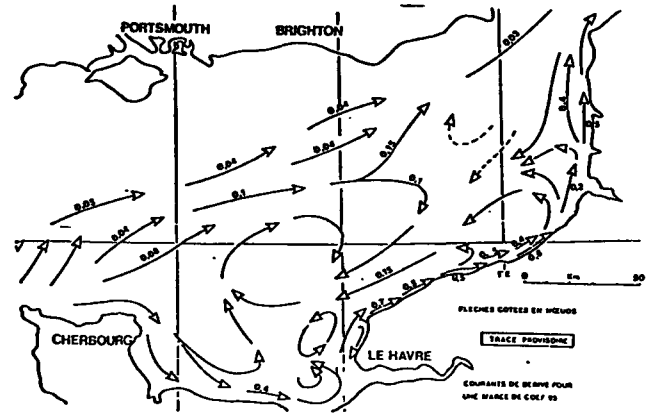

FIG. 2 Circulation résiduelle déterminée par la plaque tournante de Grenoble

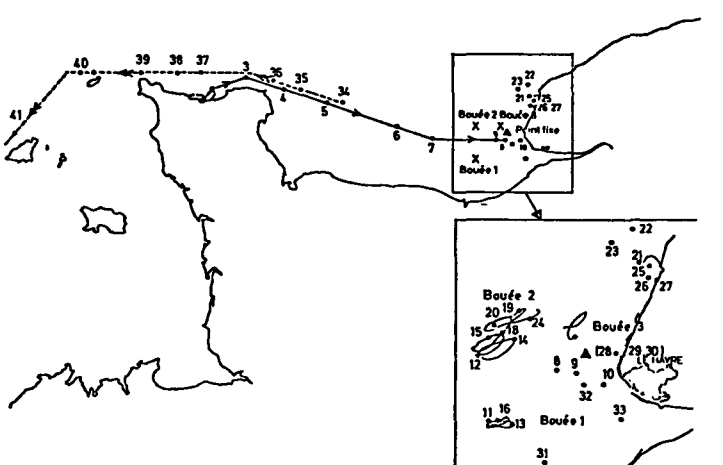

Figure 3 : Position des points d'analyse des

PUBLICATIONS

- Etude de quelques aspects du comportement de l'antimoine-125 et de son isotope stable dans les milieux marins. P. GUEGUENIAT, A. GRAUBY, R. GANDON, Y. BARON.
RAPP. après COMM. INT. Mer. MEDIT 24 - 3 - 1977

- Evolution dans les sédiments marins des radionucléides d'origine industrielles en présence d'hydrocarbures. P. GUEGUENIAT, J-P. AUFFRET, M. KHALANSKI, et J-P. DUPONT. IIe symposium Int. de radioécologie CEA. EDF. CEN. CADARACHE du 19 au 22 Juin 1979.

- Evolution de la radioactivité artificielle gamma dans des sédiments littoraux de la Manche pendant les années 1976-1977-1978. P. GUEGUENIAT J-P. AUFFRET Y. BARON. Océanologica Acta 1979. Vol. 2 p. 165-180.

- Sediments as indicators of artificial radionuclides distribution West of La Hague. P. GUEGUENIAT J-P. AUFFRET J. BALLADA.

- Donnés nouvelles sur la dispersion des radionucléides en Manche. P. GUEGUENIAT P. LE HIR. Colloque International sur l'impact des radionucléides rejetés dans le milieu marin. AIEA. Vienne 6-10 octobre 1980.

- Réunion sur le comportement des transuraniens en milieu aquatique et échange sédiment-eau. ISPRA 24-28 mars 1980.

- Etude des phénomènes d'hydrolyse du fer en milieu aqueux. R. GANDON, P. GUEGUENIAT, J. PIERI. (Aparaître)

- Etude de l'adsorption en milieux aqueux du Zn, Cb, et Mn sur divers composés minéraux du Fe^{2+} et du Fe^{3+}. R. GANDON, P. GUEGUENIAT, J. PIERI. (Aparaître)

- Etude de l'adsorption en milieu aqueux du chrome 6, du sélénium 4, de l'antimoine 5 sur divers composés minéraux du Fer^{2+} et Fer^{3+}, R. GANDON, P. GUEGUENIAT, J. PIERI. (A paraître).

Contractor: Risø National Laboratory

Contract nr: 280-79-1 B10 DK
Head of the research team: Asker Aarkrog

General subject of contract: Radioecological investigations of an environmental contamination with transuranic elements.

Title of the project No 1: Radioecological investigations of an environmental contamination with transuranic elements.

Head of Project and scientific staff: Asker Aarkrog; Karen Nilsson, Henning Dahlgaard and Heinz Hansen.
Consultants: Elis Holm, Lund University, Sweden; David N. Edgington, Wisconsin University, U.S.A.

In January 1968 the marine environment at Thule Air Base, Greenland was contaminated by plutonium and americium. Expeditions in 1968, 1970, 1974, and 1979 have studied this contamination; approximately 1 TBq ^{239,240}Pu (25-30 Ci) are present in the marine sediments. In the biota most of the contamination occurs in the benthos.

An aim of the 1979 expedition has been to make a study of the time-trend of the environmental plutonium levels, i.e. how rapidly the Pu levels decrease in the biota and in sediments. Furthermore, the vertical and horizontal distributions of the contamination of the sediments have been compared with those of previous years. The higher trophic levels have been reexamined to ascertain whether or not they are still uncontaminated, and for the first time ^{241}Am has been included in the studies.

Sediments

On the 1979 expedition sediments were collected at 17 locations to a depth of 18 cm. The sediment cores were divided into 3-cm thick layers and due to the presence of hot spots (cf. below) they were analyzed as duplicates for ^{239,240}Pu, ^{238}Pu, and ^{241}Am. Furthermore, all samples were analyzed for ^{137}Cs in order to determine the content of fallout plutonium in the samples. The horizontal as well as vertical distribution of the activities in the sediments could be described by ex-

ponential expressions. This was also the case in 1974 and a comparison between the two years was made.

The median distance increased from 5 km in 1974 to 6.5 km in 1979, i.e. the material moved 300 metres a year during this period. The median depth of the activity in the sediments increased from 4.8 cm in 1974 to 8.4 cm in 1979, corresponding to a vertical movement of 7.4 mm y^{-1}. However, the rate will probably decrease in the years to come as the vertical mixing is strongly influenced by bioturbation. The inventories by 1979 were estimated to be 1 TBq ^{239,240}Pu, 0.1 TBq ^{241}Am, 1.6 GBq ^{238}Pu, and 2 TBq ^{241}Pu. The estimate of the ^{239,240}Pu inventory was not different from that of previous years.

Approximately 9% of the samples contained hot spots, i.e. the sample deviated by more than a factor of 3 from its duplicate. The hot spots were most frequently in the upper 6-cm of sediments. Even at the most distant sediment location (50 km from point of impact) a hot spot was observed. The low value of $^{238/239,240}$Pu (= 0.020) in this sample suggested that the activity was from the accident rather than from fallout. In cooperation with David N. Edgington (Wisconsin University, USA) some preliminary studies of the plutonium speciation of the sediments have been made. It was shown that the plutonium activity in Thule sediments was soluble in sodium dithionite, which indicates that the activity in the sediments required a reducing environment before dissolution could occur.

Seawater

Eight samples of total seawater (unfiltered), 4 filters (0.45 µ) and 3 samples of filtered water were analysed for ^{239,240}Pu and ^{241}Am. The samples were all surface samples, except those collected at the point of impact. These samples were taken at 50-, 100-, 150- and 170-m depths. The sample at the greatest depth contained accident plutonium; however, all the surplus activity (52 µBq ℓ^{-1}) was present as particulates. The mean level of ^{239,240}Pu in all total water samples (except the above mentioned) was 19 µBq ℓ^{-1} (0.51 fCi ℓ^{-1}), which is comparable to the fallout level in seawater. The filtered samples indicated that approximately 10% of the plutonium activity from fallout in seawater was particulate. The mean ratio: ^{241}Am/^{239,240}Pu in seawater was 0.15 and in particulates approximately 0.3. In the sample containing accident Pu the total water showed ^{241}Am/^{239,240}Pu = 0.06 and the ratio in particulates was 0.12, corresponding to ^{241}Am/^{239,240}Pu found in sediments. We may conclude that Am is depleted in the water as compared to Pu; hence, the ratio of Am to Pu becomes lower in the water than in fallout and in particulates.

Seaplants

Sixteen samples of fucus and laminaria were analysed for ^{239,240}Pu and ^{241}Am. The mean level of all samples were 0.25 Bq ^{239,240}Pu kg^{-1} dry weight (6.8 pCi kg^{-1}). The mean value of ^{241}Am/^{239,240}Pu was 0.13. This ratio was not significantly different from that in seawater and we conclude that the seaplants collected at Thule did not show any difference with regard to uptake of plutonium or americium. The concentration factor:

$$\frac{\text{Bq } ^{239,240}\text{Pu kg}^{-1} \text{ seaplants, dry weight)}}{\text{Bq } ^{239,240}\text{Pu } \ell^{-1} \text{ seawater}} = 13 \times 10^3$$

This concentration factor was two times higher than that observed in Danish waters (6×10^3).

Benthos

Eight samples of brittlestars were analysed for ^{239,240}Pu and compared with the concentrations found in 1974. The levels were nearly unchanged. The inventory of ^{239,240}Pu in the biomass of brittlestars at Thule was 28 MBq in 1974 and 22 MBq in 1979, corresponding to an effective half-life of approximately 16 years. This is an increase in half-life compared with the period 1970-1974 when the half-life was estimated at only 2 years. However, additional samples of brittlestars should be analysed before more reliable conclusions may be drawn.

Shells of bivalves were analysed for ^{239,240}Pu and ^{241}Am. The mean ratio: ^{241}Am/^{239,240}Pu was 0.2 i.e. between that of fallout (0.3) and accident debris (0.1). The inventory in shells of bivalves was estimated at 250 MBq in 1979 as compared to 300 MBq in 1974, corresponding to a half-life of approximately 17 years. This is to be compared with a half-life of 15 years observed from 1970 to 1974. However, if the values were corrected for Pu fallout, the estimates became 130 MBq in both 1979 and 1974. In four samples of urchins the ^{239,240}Pu levels ranged between 0.2 and 0.4 Bq kg^{-1} (fresh weight) with no apparent dependence upon distance from point of impact. The mean ratio: ^{241}Am/^{239,240}Pu was 0.15.

Soft tissues of bivalves contained on the average 0.2 Bq ^{239,240}Pu kg^{-1} (7 samples) collected between 7 and 10 km from the point of impact; this is approximately one-third the value found at similar distances in 1974. However, more samples have still to be analysed before a more reliable estimate of the decrease in the plutonium inventories from 1974 to 1979 could be made.

Fish, sea birds and sea mammals

Samples of fish and sea birds were obtained during the 1979 expedition, and sea mammals were shot by local residents in April 1980.

Plutonium-239,240 (mBq kg^{-1})$_{\text{fresh weight}}$ in higher animals from Thule 1979-1980 is given below:

	Fish	Birds	Seal	Walrus
	Sea Scorpion	Brunnich's guillemot Eider		
Meat	1.6	< 0.4-3.4	< 0.4	< 0.4
Liver	< 7	2-55	< 4-2800	1-6
Bone	15	< 7-57	< 7	< 7
Roe	1.1	-	-	-

In 1974 fish showed Pu-levels in the range 4-370 mBq kg^{-1}, birds were around 14 mBq kg^{-1} and sea mammals varied between < 4 and 200 mBq kg^{-1}. The liver is apparently the organ where Pu is most likely to be detected. A single sample of seal liver showed an unexpected high $^{239,240}Pu$ concentration (75 pCi kg^{-1} ~ 2.8 Bq kg^{-1}). However, a high ratio: $^{238}Pu/^{239,240}Pu$ (~ 0.1) indicated that the plutonium source could hardly have arisen from the Thule accident. The sample will be examined further in order to identify the possible source of contamination.

Conclusion

The expedition to Thule was accomplished as planned and all abiotic samples have been analysed. The analysis of the biological material will first be completed by 1981.

Most of our initial objectives have been achieved:

1) The inventories at Thule of $^{239,240}Pu$, ^{241}Pu, ^{238}Pu and ^{241}Am from the accident were determined.
2) The amount of fallout $^{239,240}Pu$ in the marine sediments was estimated from the ^{137}Cs levels.
3) The seawater does not contain measurable amounts of Pu (or Am) from the accident, except that found in particulates just over the seabed at the point of impact.
4) The concentration factor from seawater to brown algae was the same for Pu and Am.
5) Pu and Am behave similarly in the sediments both as regards to vertical and horizontal distribution. Americium is less soluble in seawater than plutonium, hence Am/Pu is lower in seawater than in particulates.
6) The vertical displacement of the activity in the sediments has been 7-8 mm y^{-1} since the accident. This indicates that Pu and Am deposited on the seabed (by an accident) become increasingly unavailable to the biota as they are buried in the sediments.
7) Although not all biological samples have been analysed the results obtained hitherto indicate that the Pu inventories in the biota have decreased since 1974. It is, however, premature to give any figures for the transfer factors from the accidental release to the biota.
8) Plutonium from the accident was not detected in higher animals (fish, birds and mammals) with any certainty. The contamination at Thule is confined to the benthic organisms.

Publications

Asker Aarkrog, Henning Dahlgaard, Heinz Hansen, Elis Holm, Jørgen Lippert and Karen Nilsson, Provisional results from the August 1979 Thule expedition; in Environmental Radioactivity in Greenland in 1979. Risø-R-423 p. 31-42 (July 1980).

Contractant van de Commissie:

Institute of the Association EURATOM-ITAL, Wageningen, the Netherlands

Nummer van het contract: 185-76-1 BIA N

Hoofd van het (de) researchteam(s): Dr.Ir. D. de Zeeuw, subsequently Dr.Ir. A. Ringoet

Algemeen onderwerp van het contract:

- The study of various aspects of the effects of radiation on living cells making use of plant cells and related material.
- Behaviour of radiocontaminants in soils and vegetations of specific sites.

Dosimetry intercomparison studies were made to enable coordination of research on late effects of radiation, especially on partial body irradiation of rats. Lyoluminescence studies demonstrated that mannose and glutamine can be used as suitable dosimeters for intermediate doses. The clear perspex dosimeter was investigated for its dependence of oxygen diffusion, temperature and humidity during storage.

Haplopappus gracilis cell cultures were developed for cellular radiobiological studies and cell survival and mutations were studied. A very pronounced repair of potentially lethal damage was found. In *Saintpaulia ionantha* the regeneration of epidermal cells was used to study a radiation stimulated repair of potentially lethal damage for X rays and neutrons. Analysis of literature data demonstrated a close correlation between survival, utations and chromosomal aberrations. In *Oenothera organensis*, mutation studies at the S-locus suggest that low doses of radiation induce heritable changes.

A scheme to examine the possibilities for decontamination of agricultural areas after severe nuclear accidents has been prepared. The study takes into account both the short term pathway (grass - cow - milk - man) and the long term pathway (soil - grass - cattle - milk/meat - man). Numerous measures are discussed with special attention being paid to their practicability.

The possibility to fix ^{90}Sr in soil with alginates was studied. The perspectives are disappointing.

The migration of ^{90}Sr, ^{137}Cs, ^{238}Pu and $^{239,240}Pu$ in soils was studied by laboratory column elements and for ^{90}Sr and ^{137}Cs since 1957 by sampling pastures. Models to describe the migration were refined and tested. Results are satisfactory.

Soils and sediments of the river Rhine delta were analysed to determine a possible contamination of Pu and Am. Some slight enrichment could be observed; a soil of the forelands of the Rhine had a $^{239,240}Pu$ content of 500 $mBq \cdot kg^{-1}$ versus 300 mBq in a soil which was never flooded with Rhine water.

Tetren (tetraethylenepentamine) appeared to be a chemical ligand which considerably decreases the availability of transition metals in clay containing soils. The roots cultivated in a 10^{-6} molar Cu solution contained 20 times as much Cu as the roots of plants which were grown in a 10^{-6} molar Cu-tetren solution. The Cu-tetren complex once taken up by the roots, is very mobile within the plants. A drawback of using such synthetic ligands is that they do not react specifically with radioactive transition metals.

Transfer coefficients and (re)distribution of some radiocontaminants (corrosion products and transition metals) in plants have been determined on nutrient solutions and two soils. The transfer coefficients are apparently functionally dependent on the stable isotope concentration and the plant age, but independent on the water uptake at the given concentrations. The mobility of the radiocontaminants is different, being for example very low for ^{65}Zn, but fast for ^{60}Co. The transfer of ^{65}Zn from soils contaminated in their top layer is rare due to limited depth migration.

In 1974 an unexpected phenomenon was observed in our laboratories. It seemed that two isotopes of Cs, at very low concentrations, behaved differently. All samples of this experiment were stored and they were recounted during this contract period; the aberrant behaviour seemed consistent. All experiments thereafter did not show, however, any different behaviour of the two Cs isotopes.

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The meetings of the International Scientific Advisory Council (ISAC) were discontinued.

Changes in Scientific Staff

Dr. W.A.A. Bergers, Dr.Ir. L. Damen-Dellaert, Dr.Ir. F. van Dorp and Dr. J.W.G.M. Wilmer left the Institute to accept research duties elsewhere.
Ir. P. Poelstra died in July 1980.
Several guest-workers have spent 6 - 12 months at the Institute.

Resultaten van het project No. 1

Hoofd van het team en wetenschappelijke medewerkers:

K.H. Chadwick, H.P. Leenhouts, P.A.Th.J. Werry, K. Sree Ramulu.

Titel van het project:

A study of dose effect response and the inter-relationship between cell survival, mutation and chromosomal aberration in plant cells.

Beschrijving van de resultaten:

1. *Haplopappus gracilis* (Nutt.) Gray cell cultures

A. The experimental system

A replating efficiency of 80% was obtained for single cells of *Haplopappus gracilis* by using a replating technique in which small aggregates of cells were replated on top of a feeder layer. The small aggregates were grown from single cells plated at high density in soft agar on top of a feeder layer. This technique was used for survival studies.
A different technique was used for mutation studies when much larger numbers of cells needed to be treated. In this case 2 ml of cell suspension culture in stationary phase was plated on top of B-5 agar containing the selective agent cycloheximide.

B. Survival studies

a) Acute irradiation, immediate plating

Measurements of cell survival have been made using exponentially growing cells and stationary phase cells using X-rays and fission neutrons.

- Fission neutrons are more effective than X-rays and an RBE value of 3 to 5 is found, in accordance with data reported for mammalian cells.
- Cells in stationary phase are more sensitive to both X-rays and fission neutrons than cells in exponential growth.
- The plating efficiency (PE) of cells irradiated with doses of less than 4 Gy (400 rad) of X-rays exceeded that of the control cells.
- This "stimulated" PE was ascribed to an increase in cell elongation possibly caused by radiation induced endogenous cytokinine production. The increased cell growth improved the cells chance to form small aggregates in the first phase of the plating process.
- The "stimulated" PE was found to be dose rate dependent for X-rays, extending to a higher dose at lower dose rate. Acute neutrons did not give an observable "stimulation" but it was found for low dose-rate neutrons.

b) Delayed plating and decreased dose rate

The repair of both potentially lethal damage (PLD) and sub-lethal damage (SLD) was observed in the *in vitro* cultivated cells.

- Repair of PLD after X-irradiation is completed after a delay of plating of 12 hours under normal conditions; at low temperature the repair process is retarded.
- Repair of PLD after irradiation with fission neutrons follows a bi-phasic pattern with respect to the reaction

rate of the repair process and with respect to the sensitivity to low temperature.

- The stimulation effect at low dose of X-rays is not manifest when the cells - after the irradiation - are kept in condition that allows full repair of PLD.

C. Mutation studies

Mutation studies in the *Haplopappus* cells have been initiated making use of the induction of resistance to the antibiotic cycloheximide.

a) Theoretical basis of the Ch^R system

Cycloheximide is a potent inhibitor of protein synthesis: it prevents elongation of the peptide chain "growing" at the ribosome by competitively binding to the ribosome. A change in the molecular structure of the ribosome - based on a change in the DNA coding for the ribosomal structure - prevents such binding and renders the cell resistant to a concentration of cycloheximide, that would normally kill the cell by protein deprivation.

b) Dose effect relationship after irradiation with X-rays and fission neutrons

The cell killing effect and the mutation induction effect are both correlated to the applied dose in a strictly similar way. Fission neutrons are more effective in cell killing and mutation induction than X-rays, however, the net yield of mutants at the optimum dose for mutation induction is similar for both types of radiation. The optimum dose for net mutation induction is 10 Gy (1000 rad) for X-rays (3,1 $Gy.min^{-1}$ (310 $rad.min^{-1}$)) and 5 Gy (500 rad) for fission neutrons (1 $Gy.min^{-1}$ (100 $rad.min^{-1}$)).

c) The effect of delayed plating following X-rays

When the plating of the cells is delayed such that PLD repair can be completed, it appears that concomitantly with PLD repair, the mutation frequency decreases. However, since PLD repair is more pronounced than the decrease in mutation frequency, the net increase of mutants as a result of delayed plating is observed.

2. *Saintpaulia*

A technique was developed to quantify the effect of radiation on the stationary (G_0) epidermal cells at the base of several leaves. Using this technique the effect of radiation on the regeneration of these cells was found to be similar to cell survival. A dose rate effect was measured and in a fractionation experiment the repair of sub-lethal damage was found to be efficient, a first order process with time having a half-life of 2 hours.
Very little repair of potentially lethal damage was detected unless the leaves were pretreated with a small radiation dose 24 hours prior to a challenging dose. In this case the stimulated repair process enabled the cells to repair a considerable amount of potentially lethal damage. The development of the repair process was time dependent and was optimal at 12 - 24 hours after the pre-

treatment dose. The repair process was dependent on the size of the pretreatment dose and could be induced by both X-rays and fission neutrons. Analysis indicated that the repair process was induced in a single-hit process and had a neutron RBE of unity. It was shown that with delayed cultivation of the leaves after a single irradiation the repair process can develop and eventually repair some potentially lethal damage induced by the irradiation. This process is more efficient at low doses of X-rays and leads to a change in the shape of the X-ray dose response curve for regeneration which develops a flat shoulder at low doses. Very little effect on the fission neutron response curve is seen and this stimulated repair process combined with delayed plating results in an increase in the low dose RBE for neutrons from 10 for immediate cultivation to 35 for delayed cultivation. This increase in RBE is solely due in this case to a decrease in the effectiveness of the X-rays.

The induction by a low pretreatment dose of a "protective" effect in the epidermal cells correlates very closely with the effect found at the plantlet level in *Saintpaulia* previously. The plantlets are apparently derived from single regenerating epidermal cells and the results indicate that the effect found at the plantlet (organism) level can be ascribed to an effect occurring at the cellular level; it is not due to a cell competition or tissue organizational effect. The facts that plantlets are derived from single epidermal cells and that they can be grown to full plants and scored for mutations made it possible to study the effect of the stimulated repair process on the frequency of mutations. The results showed that the pretreated leaves gave a lower mutation frequency than the untreated leaves at each dose indicating that the repair of potentially lethal damage led to a reduction in the mutation frequency. However, the reduction factor for the mutation frequency was smaller than the increase in cell survival (regeneration) induced by the pretreatment and this indicates that whilst part of the repair of potentially lethal damage might be error-free, part of the repair is not error-free and still leads to mutations.

3. Analysis

In the model developed over the past eight years it is assumed that radiation induced DNA double strand breaks (dsb) are the crucial lesions responsible for biological effects such as cell reproductive death, chromosome aberrations and mutations. This assumption implies that in experiments where different biological end-points are determined from the same population of treated cells direct correlations between the different end-points should exist. The model predicts quantitative correlations between the end-points as follows:

if the number of double strand breaks (N) induced by a dose (D) of radiation is given by

$$N = \alpha D + \beta D^2 \qquad (1)$$

and p is the probability that a dsb leads to cell killing;
q is the probability that a dsb leads to a specific mutation;
c is the probability that a dsb leads to a scorable chromosomal aberration;

then cell survival $S = \exp[-pN]$ (2)

mutation frequency $M = 1 - \exp[-qN] \simeq qN$ (3)

and aberration yield $Y = cN$ (4)

N, the number of crucial lesions, is common to the equations 2, 3 and 4 and elimination of N between these equations leads to the correlative equations:

$$\ln S = -\frac{p}{q} M \quad (5)$$

$$\ln S = -\frac{p}{c} Y \quad (6)$$

$$M_1 = \frac{q_1}{q_2} M_2 \quad (7)$$

$$Y_1 = \frac{c_1}{c_2} Y_2 \quad (8)$$

Figure 1 illustrates the various straight-line correlations between the different end-points which are predicted by equations 5, 6, 7 and 8 for data taken from a small sample of suitable experiments published in the literature.

These correlations also mean that comparable effects of dose-rate, fractionation, radiation quality and sensitizers and protectors will be found for these different end-points. At low doses of radiation, where the linear (α) term in dose dominates the dose responses, the limiting relative biological effectiveness (RBE_0) will be the same for different end-points measured in the same cell population. This RBE_0 is relevant for the quality factor used in radiological protection.

The model has also been developed to provide a qualitative analysis of the synergistic interaction between ionizing radiation and other DNA damaging agents. The analysis of data on the interaction of UV and X-rays, and an alkylating nitrosourea compound (BCNU) and X-rays has shown that the experimental results are compatible with the synergistic interaction model. The quantitative analysis predicts that the interaction between a DNA damaging agent and radiation leads to an increase in the linear component of the radiation response which is significant at low doses.

4. Genetic effects of the very low doses of X-rays and fast neutrons on the S-locus of *Oenothera organensis* Munz.

The analyses on the genetic effects after treatment of pollen mother cells with low doses (0,025 - 0,2 Gy (2,5 - 20 rad) of fast neutrons and X-rays in clone III-4/55 of *Oenothera organensis* Munz. were further continued. The results showed that fast neutrons and X-rays produced significantly higher frequencies of seeds and seedlings than those obtained spontaneously (table 1). The genetic analysis of 79 progeny plants revealed that the low doses of fast neutrons and X-rays induced both permanent mutations (pollen-part) and reversible mutations at the S locus, while spontaneously only reversible mutations occurred (inspite of screening approx. 25 million pollen grains), the frequency being significantly lower than that induced by fast neutrons or X-rays. Among the 50 progeny plants derived from the treated series, so far analysed, 47 showed reversible mutations and 3 permanent mutations. Thus, the present data suggest that the low doses of radiations induce heritable changes.

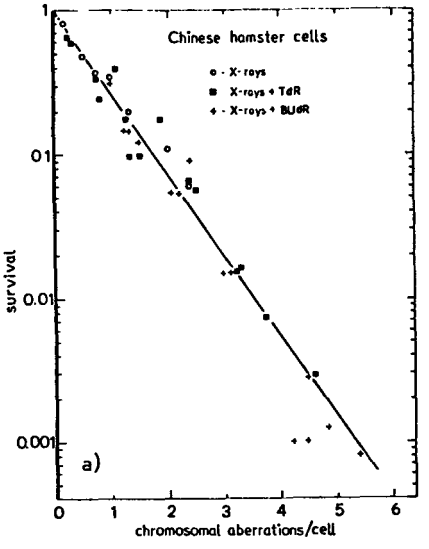
The implications of these results for Radiological Protection are:

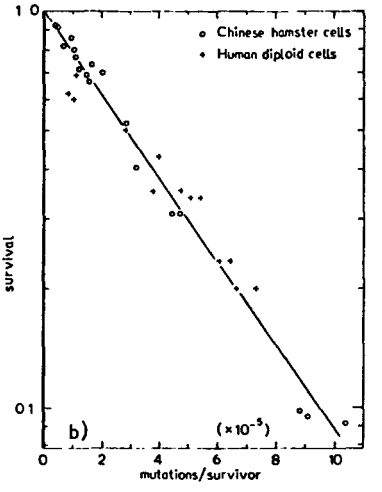
1. the repair of sublethal damage is a different process than the repair of potentially lethal damage;
2. one common type of lesion seems to be responsible for different biological end-points;
3. in general a comparable effect of dose rate, fractionation, radiation quality, sensitizers and protectors can be anticipated for different biological end-points;
4. the limiting RBE at low doses will be the same for different end points in the same cell population;
5. in testing cells radiation can stimulate a repair process which is not entirely error-free;
6. the stimulated repair can affect the low dose RBE value;
7. synergism between a chemical mutagen and radiation can lead to an increase in the linear dose component which is effective at low doses;
8. very low doses of X-rays and neutrons can induce permanent inheritable mutations.

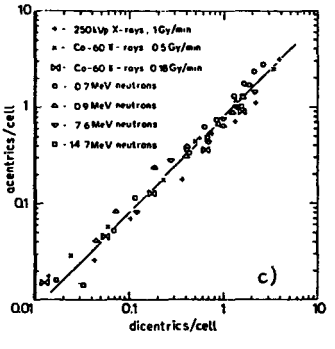
Table 1 - Frequencies of capsules and seeds after crosses between unirradiated S_3S_4 plants, used as female parents and irradiated and control S_3S_4 plants in *Oenothera organensis* Munz.

Irradiation treatments		Flowers pollinated	Capsules				Seeds			Viable seeds		
			With seeds		With viable seeds		No.	Frequency		No.	Frequency	
			No.	No. per 100 pollinations	No.	No. per 100 pollinations		Per pollination	Per million pollen grains*		Per pollination	Per million pollen grains
Control		5380	54	1,00	23	0,43	147	0,027	6,75	44	0,008	2,02
Fast neutrons												
Gy	rad											
0,025	2,5	1081	51	4,72	17	1,57	60	0,055	13,97	17	0,016	3,96
0,05	5	1056	36	3,41	15	1,42	37	0,035	8,95	15	0,014	3,63
0,1	10	889	26	2,92	11	1,24	33	0,037	9,50	11	0,012	3,17
X-rays												
Gy	rad											
0,025	2,5	787	23	2,92			32	0,041	9,09			
0,05	5	869	36	4,14			47	0,054	12,05			
0,1	10	824	29	3,52			44	0,053	11,92			
0,2	20	768	31	4,04			60	0,065	14,53			

*The mean no. of pollen grains per pollination was 4483 $\pm$ 121.







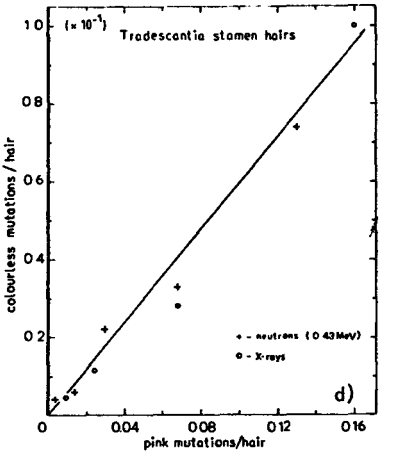


Fig. 1 - The correlations between different biological end points as predicted by equations 5, 6, 7 and 8.

a) $\ln S = -\frac{p}{c} \cdot Y$, b) $\ln S = -\frac{p}{q} \cdot M$, c) $Y_1 = \frac{c_1}{c_2} \cdot Y_2$,

d) $M_1 = \frac{q_1}{q_2} \cdot M_2$.

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Resultaten van het project No. 2

Hoofd van het team en wetenschappelijke medewerkers:

K.J. Puite and K.H. Chadwick

Titel van het project:

Development and improvement in accuracy and reproducibility of dosimetry systems for X-ray intercomparison, neutron and high level gamma irradiation.

Beschrijving van de resultaten:

1. Dosimetry intercomparison for evaluation of late effects of ionizing radiation.

A prerequisite for coordination of research programmes to study the late somatic effects of ionizing radiation in mammalian organisms is the standardization of experimental methods, such as the dosimetry. Investigations in radiation biology and radiation therapy have demonstrated that differences of 10 per cent in absorbed dose will produce clearly observable variations in biologic response. In general, cell survival analyses do not allow the prediction of variations in absorbed dose determinations smaller than 5 per cent. It has been suggested therefore that an accuracy of 5 to 6 per cent and a precision of 2 to 3 per cent is required for the determination of absorbed dose in biologic applications.

For the institutes cooperating within the European Late Effects Project Group (EULEP) intercomparisons of doses and dose distributions have been performed in 1971, 1973, 1976 and 1980. These studies have resulted in improvements with regard to the accuracy and precision of the X-ray dosimetry at these EULEP laboratories. The third and fourth intercomparison was considered to be essential for a periodical check on the dosimetry procedures and for the benefit of new groups joining EULEP in the intervening years. The intercomparisons were carried out using mailed thermoluminescent (TL) dosemeters.

The fourth dose intercomparison was organized in close cooperation with the National Institute of Public Health at Bilthoven. The dosemeters were evaluated both at this institute and at ITAL. Future work on behalf of the EULEP programme will be carried out by the Institute of Public Health.

Late effects caused by the irradiation of specific organs, e.g. lung and liver, are also studied in the EULEP research programme. For these studies collimated X-ray beams have to be employed, since it is necessary to restrict the dose to other essential organs in the animal. In connection with these programmes it was decided to initiate an intercomparison of the X-ray dose and dose distribution for partial body irradiation in 1978. The exposures at the participating institute had to be performed under conditions similar to those actually employed for rat partial body irradiations. The dose in the irradiated organs as well as the scattered doses in the phantoms have been measured and compared with the dose values from a standardization laboratory. On the basis of these partial body X-ray dosimetry studies (Fig. 1) a number of conclusions can be drawn:

(1) Dual capsules with LiF and CaF_2:Mn TL powder appear to be well suited for this type of dosimetry with X-ray beams having an HVL above 1,3 mm Cu. However, one should be aware of the disturbance caused by the capsules in the lung and liver equivalent material.

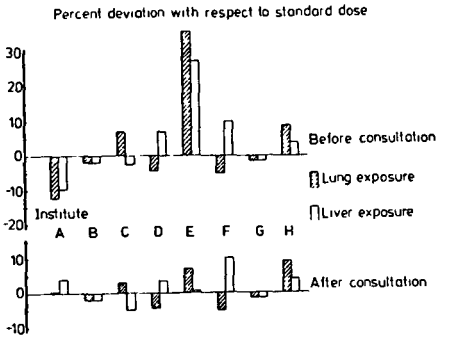

Fig. 1. Diagram showing the results of the EULEP partial body X-ray dose intercomparison before and after consultation with the participating institutes.

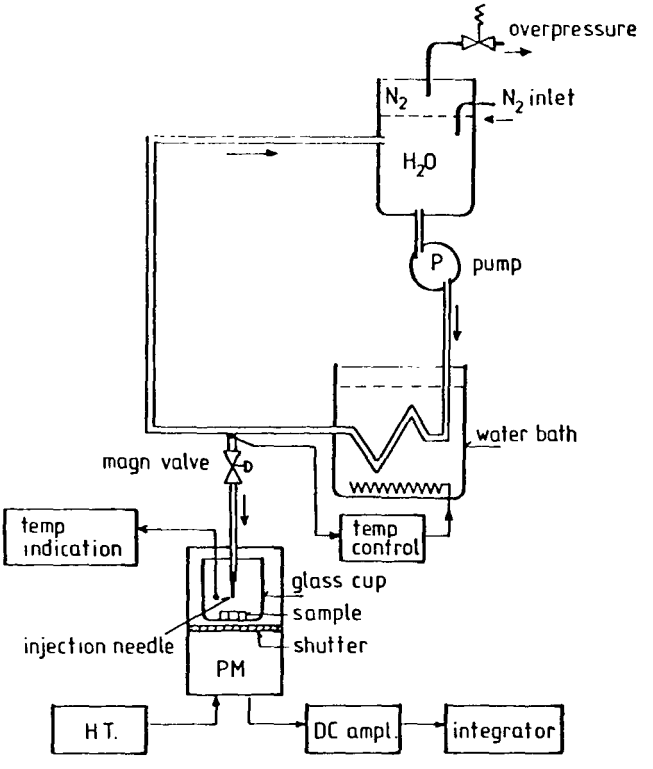

Fig. 2. Lyoluminescence apparatus.

(2) At an FSD of 50 cm large dose gradients are present in case of unilateral irradiations and minimal backscatter conditions: for example 23% for an HVL of 1,5 mm Cu in the lung substitute material and 51% in the liver substitute material. Full backscatter geometry reduces the dose gradients in the lung to 18%, but hardly influences the gradient in the liver. One should realize that the dose variation over the lung and liver (3 cm depth) will be equal to 12% due to the inverse square law alone.

(3) The scattered doses in the central part of the phantom are of the order of 1 to 5% of the dose in the primary beam, depending on the scatter material (lung or liver material) and the presence of backscatter material. The scattered dose in the distal part of the phantom amounts to 0,1 and 0,5% of the dose in the directly irradiated part.

(4) The main objective of this study was to obtain information on the accuracy of X-ray dosimetry and the adequacy of exposure arrangements for partial-body irradiations of rats. The doses delivered by the participants in the two sessions showed good reproducibility; however, large discrepancies were observed (up to 36% from the standard value). In a number of cases the sources of error were rather elementary, and it is to be expected that the results of this intercomparison will inspire the participants to perform better and more careful measurements. Four of the eight institutes generally perform partial body irradiations of rats by unilateral exposure. The large dose gradients (up to 68%) are unacceptable for radiobiological studies. Consequently for these institutes the introduction of a bilateral exposure arrangement is strongly recommended.

2. Lyoluminescence dosimetry.

- The lyoluminescence (LL) dosimetry technique is based on the production by radiation of free radicals in organic, closely tissue-equivalent, materials. Upon dissolution of these materials in water light emission can be recorded due to excited states of reaction products. This light signal is a measure of the absorbed dose in the material. Due to the low material costs, simple equipment, extended dose range and tissue equivalency, use of the LL technique in the field of radiobiology, radiotherapy, fast neutron dosimetry and high dose level dosimetry offers distinct advantages over systems in current use.

- A luminescence measuring system has been developed at ITAL which has a closed water circuit under gas pressure to carefully control the experimental conditions (Fig. 2). The monosaccharide mannose and the amino acid glutamine have been investigated as potential LL dosemeters.

- Mannose can be used in the dose range of 0,1 Gy up to 500 Gy (10 rad - 50 krad) in photon and fast neutron beams. The variation of the dose values above 3 Gy (300 rad) is 3 to 4%. The relative effectiveness of neutrons ranges from 0,34 (fission neutrons) to 0,74 (15 MeV neutrons).

- Glutamine can in principle be used in the dose range of 0,01 to 100 kGy (1 - 10000 krad). The glutamine system at ITAL has been tested for doses of 0,1 to 25 kGy (10 - 2500 krad). The variation in the dose determination is less than 5%. Therefore this dosimetry system seems to be very valuable for high-dose standardization and intercomparison for industrial radiation processing. The IAEA (International Atomic Energy Agency) has initiated research for the development of a dosemeter suitable for high dose dosimetry. During a dose intercomparison (dose range 0,01 to 3 kGy (1 - 300 krad) organized by the IAEA in 1980 the use of mailed LL dosemeters has also been tested. The results showed that the largest deviation of the dose values obtained with the ITAL glutamine dosemeters from the nominal doses was 3% for doses above 0,1 kGy (10 krad). In 1981 the lyoluminescence system will be tested in a dose intercomparison under radiation processing plant conditions using a dose range of 0,01 to 100 kGy (1 - 10000 krad).
- After 1980 no lyoluminescence dosimetry research will be performed at ITAL. The state of art of LL dosimetry will be described in a joint review article together with Dr. K.V. Ettinger of the University of Aberdeen. Although no LL dosimetry readers are available commercially it is possible to use some existing commercial apparatus after simple modifications e.g. the LKB Luminometer 1250 and the Packard Pico-Lite Luminometer.

3. Clear Perspex Dosimetry

The influence of two environmental factors, temperature and humidity, on the stability, reproducibility and accuracy of the clear perspex dosemeter system have been studied. The results can be summarized briefly:

- oxygen diffusion which causes a fading of the radiation induced absorbance A (optical density, OD) at all wavelengths is temperature dependent. It proceeds more rapidly at higher temperatures. The speed of oxygen diffusion is also dependent on the batch of perspex;
- when the diffusion of oxygen is prevented by treatment in vacuum the conversion of the induced free radical species R_1 to the secondary free radical species R_2 can be studied. R_1 and R_2 have different optical absorption spectra and the R_1 to R_2 conversion alters the optical density spectrum, in general low wavelengths increase and higher wavelengths decrease. It has been found that the R_1 to R_2 conversion is also temperature dependent and proceeds more rapidly at higher temperatures. At 45 °C under vacuum the cross-over wavelength caused by the spectral change is not constant but is dose dependent, under these conditions of storage it is not possible to select one wavelength for stable OD measurement and accurate dose estimate;
- on storage in air at different temperatures a reasonably stable cross-over wavelength has been found for at least 24 h after radiation at 35 °C. Dose estimates at this wavelength can be made with 3 - 5% reproducibility at 99% confidence limits and an absolute accuracy of less than 5% should be achievable even when irradiation takes place at 45 °C. This is not true for 305 nm measurements made 24 h after radiation, the OD at this wavelength at 24 h is very temperature dependent and errors of 10% can easily be made;

- irradiation at 45 °C for 16 h to a total dose of 35 kGy (3,5 Mrad) indicated no oxygen diffusion induced fading during irradiation and the OD was the same as 35 kGy (3,5 Mrad) given at 20 °C in 3 h when measured at 314 nm. This indicates that the OD-dose relationship remains constant for irradiation temperatures up to 45 °C and irradiation times of less than 20 h when measurements are made at 314 nm;
- the newer batch 5 of the HX dosimetry perspex is thicker (1,75 mm), has a slower O_2 diffusion induced fading and demonstrates no build up of OD at 305 nm after a short irradiation at room temperature. Irradiation at 45 °C for 10 h or 20 °C for 3 h to the same dose gave the same OD at 314 nm but a different OD at 305 nm. This means that although the OD at 305 nm is more stable after irradiation prolonged irradiation at higher temperatures can lead to errors if dose estimates are made at 305 nm. At 314 nm the OD remained constant for 9 days at 45 °C under vacuum. The new batch 5 HX perspex is a more stable dosemeter than the previous batches;
- perspex takes up water to 2% in weight, the increase is initially quite rapid even at room temperature but proceeds more rapidly at higher temperatures. At 20 °C saturation takes several days, at 80 °C saturation takes 48 h. The water molecules are absorbed as quickly from water vapour or water;
- comparison of the dose response of samples saturated at different levels of relative humidity at 20 °C has demonstrated very little differences from 0% RH to 100% RH water vapour although the higher RH samples tended to give a slightly higher OD reading. Samples saturated in water at 80 °C for 48 h prior to irradiation showed a radically different radiation response above 10 kGy (1 Mrad) the dose curve saturating rapdily;
- measurements with samples saturated with water at 20 °C, at 80 °C for 48 h or treated at 80 °C for 48 h in air have revealed that it is neither the water saturation nor the 80 °C treatment but the combination of water and 80 °C that leads to the alteration in the dose response relationship and that this treatment mainly reduces the unstable free radical component of the induced optical density. Since pre-saturation of perspex with water at 80 °C is unlikely to occur in practice it can be concluded that humidity differences should not affect the response of the clear perspex dosemeter in practical applications.

Since 1977 the institute has cooperated in the IAEA High-Dose Standardization and Intercomparison Programme by irradiating several dosemeter packages to known doses in intercomparison trials and providing consultant expert advice on programme development.

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Resultaten van het project No. 3

Hoofd van het team en wetenschappelijke medewerkers:

F. van Dorp, M.J. Frissel, P. Poelstra, A. Ringoet

Titel van het project:

Scheme for decontamination of agricultural areas after severe nuclear accidents

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Beschrijving van de resultaten:

In 1980 this project was completed by preparing the final report *"Agricultural measures to reduce radiation doses to man caused by severe nuclear accidents"*, which will be published early 1980. This report contains all the information collected in the period 1976-1980. It is divided into three parts. The first part describes models to calculate radiation doses to man from deposited radionuclides via agricultural pathways. The second part describes possible measures to reduce the radiation dose to man, their limitations and their practicability. The last part describes fields for future research. A summary of this report is included in the following pages.

Experimental work in 1980 has been carried out in cooperation with Dr. D. van der Borght (CEN/SCK, Mol, Belgium) concerning temporary fixation of ^{90}Sr in soil with alginates. Alginates in combination with Sr ions form insoluble compounds, therefore it was expected that alginates could immobilize ^{90}Sr in soil. Experiments with undisturbed soil columns showed, however, that the alginates are decomposed by microbial activity in soil. Therefore the perspectives for the application of alginates are disappointing. The results of this experiment are also presented in the following pages.

F. van Dorp, R. Eleveld, M.J. Frissel

AGRICULTURAL MEASURES TO REDUCE RADIATION DOSES TO MAN CAUSED BY SEVERE NUCLEAR ACCIDENTS

Agricultural land and products may become contaminated after a severe nuclear accident. If radiation doses to man caused by the ingestion of contaminated agricultural products from such areas will be unacceptably high, measures to reduce this radiation dose will have to be taken. Radiation doses to man can be estimated by using models which describe quantiatively the transfer of radionuclides through the biosphere. The following processes and pathways are described in this study:

- *accidental releases into atmospheric environments and subsequent nearby deposition (= not world wide fallout)* (atmospheric dispersion and deposition models are not included in this study)

- *contamination of crops by direct deposition and the subsequent short term pathway (e.g. grass-cow-milk-man)*
- *contamination of soil and the subsequent long term pathway (e.g. soil-crop-man, soil-grass-cattle-milk/meat-man).*

Depending on the degree of contamination (to be predicted by atmospheric dispersion models, or to be determined by direct measurements), and depending on the estimated radiation doses to man (to be calculated by using the models described in this study) measures can be advised. The most important measures are the following.
Short term measures:

- *feeding cattle with uncontaminated food if possible*
- *production and storage of milk powder when the contamination consists of ^{131}I;*
- *administration of bentonite to cattle to reduce the uptake of cesium from feed;*
- *administration of calcium to cattle to reduce the uptake of strontium from feed;*
- *decontamination of milk by removal of strontium and cesium on ionexchange columns.*

Long term measures:

- *removal of contaminated crops if present;*
- *removal of the contaminated top layer of the soil, applicable only on stable and smooth soils;*
- *application of lime or gypsum to reduce the uptake of Sr by crops from soil;*
- *producing crops with a low transfer of radionuclides from soil to edible products;*
- *reduction of the radionuclide content in the final consumable products by processing treatments.*

Special attention has been paid to the practicability of the measures. A cost-analysis, however, was not carried out.

Some of the fields for future research are:

- *the improvement of transfer models;*
- *the determination of realistic parameters to be used in these models;*
- *the study of more efficient methods to measure contamination levels in the field;*
- *the study of the effects of application of some complexing agents to soils or cattle to reduce the transfer of radionuclides;*
- *the study of product treatments which reduce the radionuclide content of the final product.*

O. van der Borght, M.J. Frissel, P. Poelstra, D. Bannink

TEMPORARY FIXATION OF ^{90}Sr IN SOIL WITH ALGINATES

Alginates react easily with Sr ions forming insoluble compounds. Therefore it was expected that alginates could temporarily immobilize ^{90}Sr in soils. Alginates are extracted from seaweed and consist mainly of polyguluronic acid and polymannuronic acid. The Na-form is soluble in water. A 4% solution in water is somewhat viscous, but still easy to handle, so from a technical point of view the method seemed promising.

An experiment has been carried out on four soil columns. Two soil columns were treated with alginates, two were used as controls. The soil was a sandy soil with a low organic matter content, so a soil in which ^{90}Sr migration is rather fast compared to other soils.

The experiment was carried out in the ITAL soil column installation (Bannink et al., 1977); the main characteristics of this installation are that the soils are not saturated with water and that artificial rain is supplied as droplets by a rain simulator. The length of the columns was 100 cm, the diameter 12 cm and the soils were undisturbed.

At the beginning of the experiment the soils were brought to water equilibrium (i.e. efflux equals influx). Thereafter the soil surface was contaminated with $[^{85}Sr]Cl_2$ by applying a carrierfree solution of 10 ml (0,1 ml cm^{-2}). After 24 h 113 ml of a 4% alginate solution (1 ml cm^{-2}) was supplied. Again after 24 h, leaching started by applying 1 cm of artificial rain (composition rain $6,9 \times 10^{-5}$ N $CaCl_2$, $2,3 \times 10^{-5}$ N NaCl, $2,3 \times 10^{-5}$ N KCl, acidified with H_2SO_4 to pH 4,5). For half a year the columns were scanned every 14 days with a soil column scanner. The results of the scannings after 16 and 184 days are shown in fig. 1. After 16 days there is a slight indication that the ^{85}Sr in the columns with alginates moved a little faster than in the columns without alginates. Because the experiments were carried out in natural, undisturbed, soils, such slight differences can also be caused by natural differences between the soil in the different columns. After 168 days a real difference became clear: in the columns with alginates the migration was delayed by a factor of about 1/3, compared to the controls; increased tailing counteracted this effect.

Additional observations were: measurement of the redox potential within the column 10 cm below the soil surface and measurement of the pH of the effluent. These observations showed that in the columns without alginates no significant changes of redox potential or pH occurred, indicating no important microbiological activity. In the columns with alginates the redox potential dropped from about 730 mV to about 500 mV, while the pH of the effluent increased from 4,6 to 6 - 7. This indicates significant microbiological activity. It must therefore be assumed that alginates were decomposed. This would explain the small differences between the two sets of soil columns. The tailing which was observed in the columns with alginates may be ascribed to small quantities of complexing agents which were formed during the decomposition of the alginates.

So far, the perspectives for the application of alginates for the temporary fixation of ^{90}Sr on soils seem disappointing. Of course it could be attempted to sterilize the soils, but in practice such a sterilization is not possible.

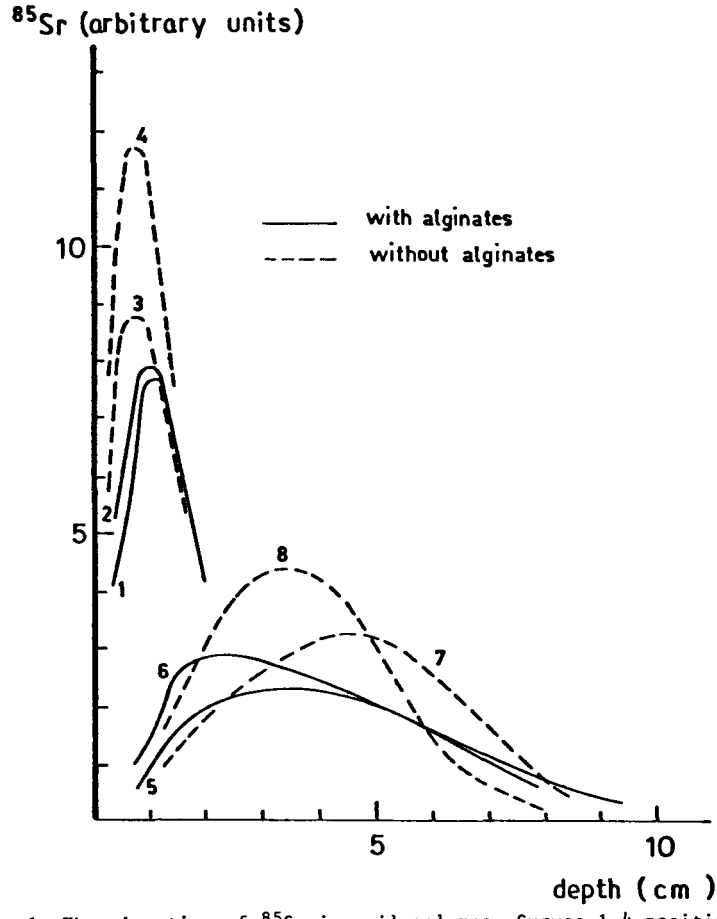

Fig. 1. The migration of ^{85}Sr in soil columns. Curves 1-4 position of ^{85}Sr after 16 days of leaching, curves 5-8 position after 184 days of leaching. The total amounts of ^{85}Sr differ slightly from column to column. Amounts of ^{85}Sr are corrected for decay, therefore they are expressed as arbitrary units.

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Resultaten van het project No. 4

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra, N. van der Klugt, F. van Dorp.

Titel van het project:

Migration of ^{90}Sr, ^{137}Cs and Pu in soils. Verification of a computer model on the behaviour of these radiocontaminants in soils of Western Europe.

Beschrijving van de resultaten:

This project was based on and is partly a continuation of an earlier project which started in 1957. From that time on four pastures were surveyed for their ^{90}Sr content. Four layers at depths of 0-5, 5-10, 10-15 and 15-20 cm were analysed. From these field data and from laboratory column experiments a model was derived to predict the behaviour of ^{90}Sr in soil. Gradually the number of sampling sites increased and also attempts were made to survey ^{137}Cs.

At this stage of the investigations the 1976-1980 project started. Aims were:
1. Verification of the ^{90}Sr model.
2. Development of a simple method to determine ^{137}Cs. Development and test of a model for the behaviour of ^{137}Cs in soil
3. Development of methods to determine ^{238}Pu and $^{239,240}Pu$ in soil. Development and test of a model for the behaviour of Pu in soil.

In general it can be stated that all these aims have been achieved.

Further a method to determine ^{241}Am (at fallout levels) is also in the process of development.

During the course of the project 25 different sampling sites were used, but only a limited number of sites could be continuously used during the whole period from 1957 until the present.
The results from 14 sites, together with simulation results have been described in the report "Modeling of the transport and accumulation of the radionuclides of Sr, Cs and Pu in soil. Experimental verification by Frissel, Jakubick, Van der Klugt, Pennders, Poelstra and Zwemmer, which will appear early 1981. Table 1 summarizes the data for Pu and Am.
Three representative graphs of the concentration of ^{90}Sr, ^{137}Cs and $^{239,240}Pu$ in the course of time are shown in the Figures 1, 2 and 3 respectively. These figures show also the simulated results, i.e. the curves which were calculated using the models. There are, however, considerable differences in the degree of sophistication of the models for Sr, Cs and Pu. The model for Sr is a simulation model. In principle all parameters can be obtained from independent measurements. In practice it appears that some adaptation is necessary.
The models for Cs and Pu are pseudo residence time models. The reason why these simpler models were used was the difficulty in predicting irreversible adsorption/desorption phenomena. Relevant data for the model have therefore to be determined from the site under study. In fact the model is a kind of extrapolation model.

A literature study showed that observed data for ^{90}Sr and other theories confirmed the model developed. For ^{137}Cs there are less data available, but there is no reason to assume that the model is not generally

applicable. For $^{239,240}Pu$ the number of observations in soil is very limited, only part of the existing data support the residence time model. A study of Pu chemistry shows that so many complications may occur that it must be assumed that in numerous situations the model will not be applicable.

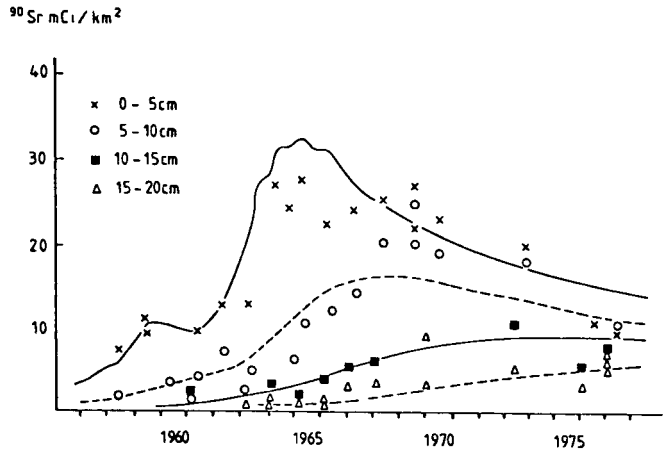

Fig. 1. ^{90}Sr activity concentration in a mucky peat soil near Groot Ammers. Dots refer to observations. Curves to calculations based on a simulation model. The upper curve refers to layer 0-5 cm, the second one to layer 5-10 cm, etc.

Table 1 - Concentration of Pu and Am in the upper layer of three soils. Mean values mBq/kg (pCi/kg)

Location	Groot Ammers				Hooglanderveen				Schoonebeek			
Nuclide	$^{239,240}Pu$		^{241}Am		^{238}Pu		$^{239,240}Pu$		^{238}Pu		$^{239,240}Pu$	
Depth cm	mBq/kg	pCi/kg	mBq/kg	pCi/kg	mBq/kg	pCi/kg	mBq/kg	pCi/kg	mBq/kg	pCi/kg	mBq/kg	pCi/kg
0 - 5	655	17,7	340	9,1	22	0,6	240	6,4	7	0,2	800	21,6
5 - 10	400	10,8	190	5,1					30	0,8	560	15,1
10 - 15	110	2,9	100	2,7	11	0,3	126	3,4	11	0,3	130	3,5
15 - 20	26	0,7	70	2,0	4	0,1	11	0,3	4	0,1	15	0,4
20 - 25	26	0,7					7	0,2	7	0,2	22	0,6
25 - 30	11	0,3					7	0,2	4	0,1	7	0,2
30 - 35	26	0,7					7	0,2	7	0,2	<4	<0,1
35 - 40	18	0,5					4	0,1				
40 - 50	15	0,5					7	0,2				
50 - 60	7	0,2					7	0,2				
60 - 70	4	0,1										
70 - 80	4	0,1					4	0,1				

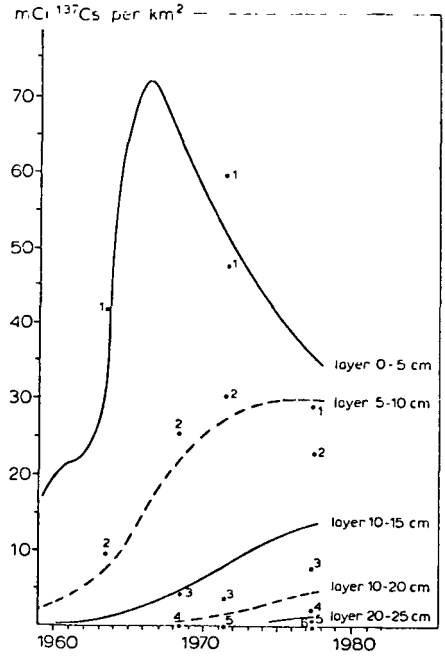

Fig. 2. ^{137}Cs activity concentration in a mucky peat soil near Groot Ammers. Dots refer to observations. Curves refer to calculations; pseudo residence time 3 $a \cdot cm^{-1}$.

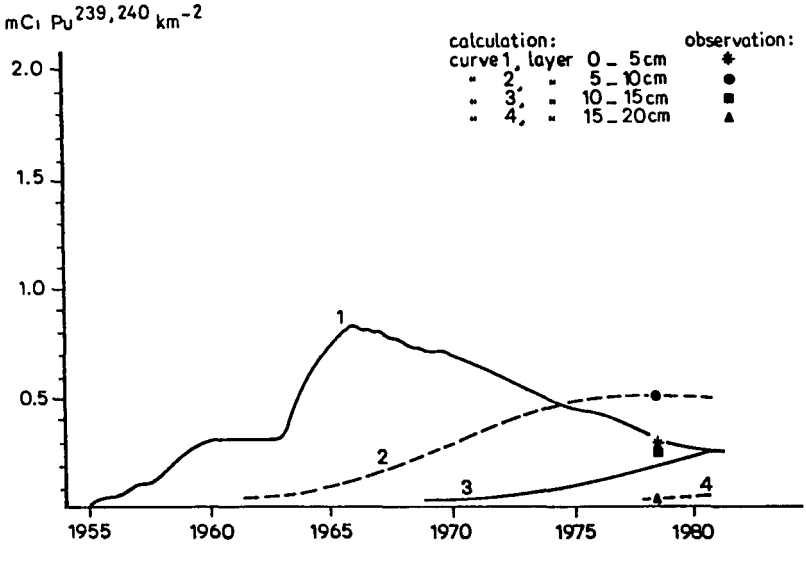

Fig. 3. $^{239,240}Pu$ activity concentration in a podsol soil from Hooglanderveen. Dots refer to observations. Curves refer to calculations; pseudo residence time 2,5 $a \cdot cm^{-1}$.

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Resultaten van het project No. 5

Hoofd van het team en wetenschappelijke medewerkers:

P. Poelstra, N. van der Klugt, M.J. Frissel

Titel van het project:

A survey of the contamination of plutonium, americium and radioactive metals in soils of the Rhine river delta.

Beschrijving van de resultaten:

The first years of the contract period were utilized to develop reliable methods to determine α-emitters at fallout and waste out levels. Two problems had to be solved: 1. The chemical separation of Pu and Am from the sediment samples and 2. The development of suitable counting techniques. The determination of Pu has been reliable since 1979, the Am determination is in development. In the last years attention was mainly focussed on the determination of Pu and to a lesser degree of Am in sediment samples of the Rhine river. Samples were taken from a clay-type soil from the forelands of the river Rhine near Valburg. This area is from time to time flooded by water of the Rhine. During such floods considerable deposition of clay and other particles occurs. Because it must be assumed that almost all Pu and Am is adsorbed on clay and other particles, the Pu content of the soil will reflect the presence of Pu and Am in the river. At Valburg five sites were sampled. Differences in Pu content may be caused by analytical difficulties and/or actual differences.

At the moment the standard deviation of the $^{239,240}Pu$ data when determined from one (homogenized) soil sample and with the same chemicals and ^{236}Pu standard is 45 mBq/kg (1,2 pCi/kg) for levels of 0,5 Bq/kg (15 pCi/kg). (^{236}Pu recovery varies, depending on soil type, between 30 and 80%). The reported differences must therefore be attributed to actual differences of Pu levels.

The second sampling location was in the Biesbosch, an area consisting of reed banks, old and recent river deposites, creeks and streams. Four sites were sampled, some consisted of clay/sand mixtures, others contained almost only organic matter. In another research programme we had found remarkable accumulation of heavy metals at some of these sampling sites. Therefore we expected similar results for Pu and Am.

A third sampling site was taken outside the delta, namely a sandy soil near Amersfoort. This area has never been flooded and is used as a reference. Results for all three locations are shown in the tables 1 - 3. From the tables it appears that the $^{239,240}Pu$ content of the upper soil layer from Valburg has a somewhat higher Pu content than those from Amersfoort (500 mBq/kg (13,4 pCi/kg) versus 300 mBq/kg (8,06 pCi/kg), mean values for the 0-5 cm layer). Pu content decreases with depth in both soils, but the difference between the soil remains (for example in the 20-25 cm layer 70 versus 11 mBq/kg (1,9 versus 0,3 pCi/kg)). A slight enrichment of Pu in the foreland soils of the Rhine might therefore be present. The enrichment of Pu and Am is certainly less than that of heavy metals such as Hg.

The values for the Biesbosch are again slightly higher than for Valburg. Pu is also observed at greater depth (610 mBq/kg (16,4 pCi/kg) for the upper 40 cm) and considerable enrichment seems to occur. It can, however, not be concluded that considerable accumulation occurred. The density

of the sediment from the Biesbosch is only one quarter of that of the soil from Amersfoort. Therefore the enrichment expressed per unit surface area is only 1,2. Because of the great heterogeneity of the Biesbosch sediments this value has an indicative meaning only.

Further research on possible accumulation of transuranium elements in the Rhine and Scheldt deltas will be carried out in cooperation with the Delta Institute at Yerseke.

Table 1 - Pu and Am contents of soils from Valburg

Radionuclide:	^{238}Pu			$^{239,240}Pu$				^{241}Am	
Sampling site:	1	2	3	1	2	3	5	4	5
Sampling date:	1/78	4/78	4/78	1/78	4/78	4/78	10/78	4/80	10/78
Depth cm	$mBq \cdot kg^{-1}$			$mBq \cdot kg^{-1}$				$mBq \cdot kg^{-1}$	
0 - 5	44	26	26	600	415	445	420	120	160
			30	560		415	435		240
				630					
				560					
5 - 10	30	26	26		545	545		70	
			30			545			
10 - 15		18	18		125	145		38	
			18			125			
15 - 20		7	7		70	78	74	21	
			7			70			
20 -25			15			78	33		
			7			96			
25 - 30							30		

Table 2 - Pu content of sediments from the Biesbosch

Radionuclide	^{238}Pu			$^{239,240}Pu$			
Sampling site:	2	3	4	1	2	3	4
Sampling date:	2/78	2/78	3/78	4/76	2/78	2/78	3/78
Depth cm	$mBq \cdot kg^{-1}$			$mBq\ kg^{-1}$			
0 - 5		48				560	
		63				555	
		63				550	
						600	
0 - 20	56				850	475	
	59				775	465	
	52				805	545	
	53				850	470	
0 - 10			56				460
10 - 20			>56				>460
20 - 30			33				700
30 - 40			22				610
40 - 50			11				220
50 - 60			7				81
60 - 70			11				48
70 - 80			7				52

Table 3 - Pu and Am content of a soil from Amersfoort

Radionuclide:	^{238}Pu		$^{239,240}Pu$		^{241}Am
Sampling site:	1	2	1	2	2
Sampling date:	5/77	11/79	5/77	11/79	11/79
Depth cm	$mBq \cdot kg^{-1}$		$mBq \cdot kg^{-1}$		$mBq \cdot kg^{-1}$
0 - 5	22	7	310	255	96
	22	15	365	237	
	22		285	314	
	26		335		
5 - 10			330	92	141
			330		
10 - 15	11		155	66	70
	11		150		
15 - 20	7		15	7	15
	4		11		
20 - 25			4	11	
			4		
25 - 30			7	7	
			4		
30 - 35				7	
35 - 40				4	
40 - 50				7	
50 - 60				7	
70 - 80				4	
80 - 100	< 4		< 4		

Publications

Frissel, M.J., Dorp, F. van, Poelstra, P. Use of residence time models in ecological studies of transuranics. Proceedings IAEA-CEA meeting "Behaviour of transuranics in the aquatic environment and sediment-water exchanges", ISPRA, (1980).

Resultaten van het project No. 6

Hoofd van het team en wetenschappelijke medewerkers:

J. Sinnaeve, F. Smeulders, M.J. Frissel, A. Ringoet, P. Poelstra

Titel van het project:

Immobilization and reduction of the availability of radiocontaminants in soils.

Beschrijving van de resultaten:

The aim of the project was to investigate to what extent synthetic ligands could be used to reduce the availability of radiocontaminants for plant uptake and the percolation to groundwater. A polyamine-type ligand, tetraethylene-pentamine (tetren or T), was used. It forms stable complexes with some transition metals, to which some radiocontaminants belong. The complex obtains an extra-stabilization when adsorbed on alumino-silicates. The basic assumptions underlying the extra-stabilization effect were described *in extenso* in the 1976 annual report. Throughout the study, a pure clay mineral, illite, characteristic clay mineral of most West-European soils, a sandy-loam soil from Horst, The Netherlands and a clay soil from Winsum, The Netherlands, have been used.

A. Soil physico-chemical aspects

Data of adsorption experiments are given as isotherms and as the natural logarithms of the stoichiometric equilibrium constant as a function of the adsorbed fraction. Figure 1 illustrates the effect of complex formation with tetren on the ion exchange selectivity of Zn, Ni, and Cd in the Winsum soil. These results are similar to those found for Cu (partly depicted (Fig. 1)). In the absence of tetren, a high selectivity is observed at low coverage: the selectivities decrease in the order Cu > Zn > Ni > Cd. The tetren-complex selectivity data roughly correspond to a two-order-of-magnitude increase of the stability constant.
Figure 2 illustrates the results obtained with the Horst soil. In contrast to what is observed for the Winsum soil, complex formation with tetren results in a shift of the metal (-complex) into the liquid phase, the effect becoming more pronounced with increasing coverage. This indicates that the stability of the tetren complexes is higher than the stability of the metal-humus complexes. Evidently, if the metal complex is less retained by the humic fraction and if only minor amounts of clay are available, the equilibrium concentration levels of the complex in the liquid phase are bound to increase.

B. Stoichiometric aspects of the uptake of Cu^{++} and Cu-tetren by plants

Maize plants (*Zea mays* L. cv. spec.) were grown for three weeks on a complete Hoagland-Arnon I (HA-1) nutrient solution. After preconditioning during 24 hours on a two times refreshed nutrient solution without microelements, the plants were transferred to a 10 times diluted HA-1 solution without microelements but containing 10^{-6} mole/l $Cu(NO_3)_2$ labelled with ^{64}Cu or 10^{-6} mole/l Cu-tetren labelled with ^{64}Cu and ^{14}C-marked tetren. The plants were harvested after an uptake period of 26 hours (of which 13 hours light). The results are summarized in table 1.

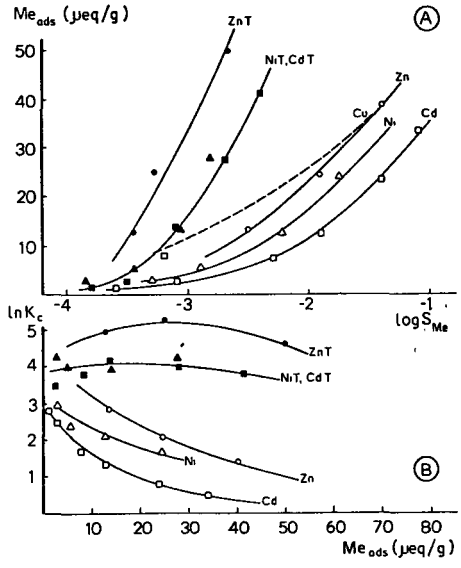

Fig. 1. A. Ion exchange isotherms for the Cd T-Ca, Zn T-Ca, Ni- T-Ca and Cd-Ca, Zn-Ca, Ni-Ca pairs of the Winsum heavy clay soil.
B. Selectivity coefficients for the exchanges Cd-Ca, Zn-Ca and Ni-Ca in the absence and presence of tetren as a function of the metal ion surface composition.

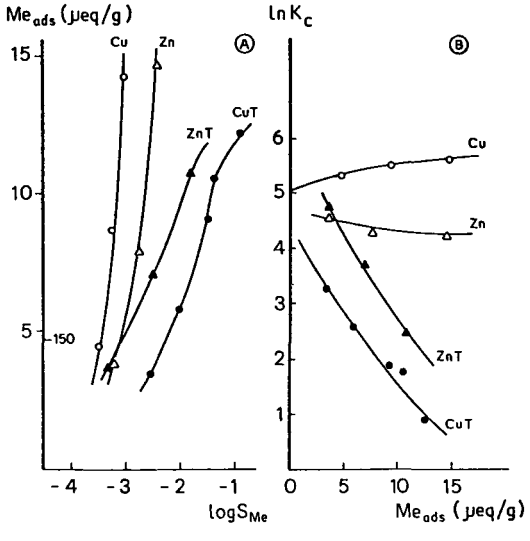

Fig. 2. A. Ion exchange isotherms for the Cu T-Ca, Zn T-Ca and Cu-Ca, Zn-Ca pairs of the Horst sandy loam soil.
B. Selectivity coefficients for the exchanges Cu-Ca and Zn-Ca in the absence and presence of tetren as a function of the metal ion surface composition.

Complex formation leads to a marked decrease of the Cu-content of the roots (195 µmole/kg versus 3,6 mmole/kg, the reverse being observed for the stem (39 versus 3,4 µmole/kg) and for the leaves (9 versus 0,4 µmole/kg).

Table 1 - Stoichiometry of the uptake of Cu and tetren supplied as Cu T 10^{-6} mole/l and comparison with the uptake of Cu supplied as $Cu(NO_3)_2$ 10^{-6} mole/l by 3 weeks old maize plants after a 26 hours absorption period. Mean values (and standard deviation) for 3 replicates in nmole·g^{-1} per 26 h.

HA-1 1/10 solution without microelements	Cu-tetren 10^{-6} mole/l			$Cu(NO_3)_2$ 10^{-6} mole/l
Plant organ	Cu	tetren	tetren/Cu	Cu
Roots	195 ± 23	167 ± 15	0,86 ± 0,13	3645 ± 641
Stem	39 ± 2	37 ± 4	0,94 ± 0,11	3,43 ± 0,6
Leaves	9 ± 3	8 ± 2	0,87 ± 0,35	0,43 ± 0,1

The ratio tetren/Cu is only slightly different from unity and this provides evidence that Cu enters the root system as a Cu T complex and because of the higher stem and leaves contents, it is also translocated as such. *In vivo* measurements of the ^{64}Cu translocation with semi-conductor radiation detectors placed along the stem revealed that translocation of the Cu-tetren was five times higher. This was also evidenced by the distributions of Cu^{++}, Cu T^{++} and TH_3^{3+} (protonated tetren) in a papyrus stem (*Cyperus papyrus* L. cv. spec.), to which labelled solutions were supplied for 330 minutes. Copper supplied as $Cu(NO_3)_2$ concentrated in the lower part of the stem whereas chelation of the Cu ion resulted in a lower concentration at the base of the stem and a distribution over its length (figure 3).
The mobility of the trivalent TH_3^{3+} is between that of Cu^{++} and that of Cu T^{++}.

C. Growth experiments using a natural clay soil

The final growth experiments, using the heavy clay soil (Winsum) were carried out in the Experimental Soil Plant Atmosphere System (ESPAS) at ITAL. Four treatments were considered: 1) the control soil, b) a Cu contaminated soil (9,5 meq/kg or 300 mg/kg), c) a Cu T treated soil (9,5 meq/kg Cu with a molar ratio tetren/Cu equal to 1,5) and d) a tetren treated soil (7,1 mmole/kg, i.e an equal amount of tetren as in treatment c). Maize seeds were germinated directly in soil columns and after 20, 34, 44 and 55 days respectively, three plants were harvested. The yields of root and shoot are depicted in figure 4. It is readily seen that chelation of Cu neutralizes metal toxicity. Comparison of the yield data of control and tetren treated soil shows a lower relative growth of the latter up to 35 days and a higher relative growth in the following period. This was due to a delayed senescence of the oldest leaves of the plants growing on the tetren treated soil. The application of tetren on a copper contaminated soil resulted in a drastic decrease in the copper concentration of the root (175 versus 430 mg/kg) and a normal concentration in the shoot (10 versus 21 mg/kg). Unchelated tetren decreased the Cu concentration of the roots; Zn and Mn concentrations increased but the iron concentration was hardly

modified. This demonstrates the very beneficial effect of tetren at toxic concentrations of transition metals (e.g. Cu, Co, Zn) whereas no deficiency symptoms occur at trace levels.

D. Biodegradation of chelated and unchelated tetren in soils

In the clay soil (Winsum), the total amount of $[^{14}C]O_2$ recovered after 1 year, suggested an overall degradation of about 20% of the chelated tetren. The decomposition of unchelated tetren was much faster and reached about 50% after 1 year. The extra-stabilization effect of the chelated form may account for this. In both cases, increasing humidity and higher temperature accelerated the breakdown. The presence of a root microflora also enhanced the degradation. In soil columns with a dense root system, the $[^{14}C]O_2$ recovery was 6,2 and 5,2% for Cu T and T respectively, whereas without cropping it was 1,2 and 1,5%. In the sandy loam soil, the rate of degradation was much faster. After 1 year, the amount of $[^{14}C]O_2$ recovered indicated a total degradation of about 70% per year.

E. *In situ* experiment

A field experiment on a Zn-contaminated soil (about 100 mg/kg Zn in the top layer) is in progress. The first results indicate a favourable effect of a tetren treatment on plant growth and the treatment eliminates Zn toxicity symptoms. The preliminary results confirm the conclusions drawn from the above reported laboratory experiments.

F. General conclusion

In the light of the reported results, some important conclusions may be drawn.

1) Due to the low chemical concentrations of radiocontaminants even after accidental contamination and due to the presence in the soil of carrier ions, it is impossible to immobilize the radiocontaminant in a selective way.
2) In cases of local contamination, complexing agents of the polyamine type (e.g. tetren plus clay mineral) may be used as a strong "temporary sink" for stabilizing the situation in order to prevent further spreading before adequate measures (e.g. removal of the top soil layer) can be taken.
3) Complexing agents, although not suited to reduce completely the availability of radiocontaminants for uptake by plants, may be used to reduce the soil-plant transfer without provoking nutrient deficiencies.
4) In case of contamination of agricultural land, the appropriate measures to be taken must be evaluated in the light of the local circumstances. The use of complexing agents can contribute to limit the collective dose commitment by ingestion.

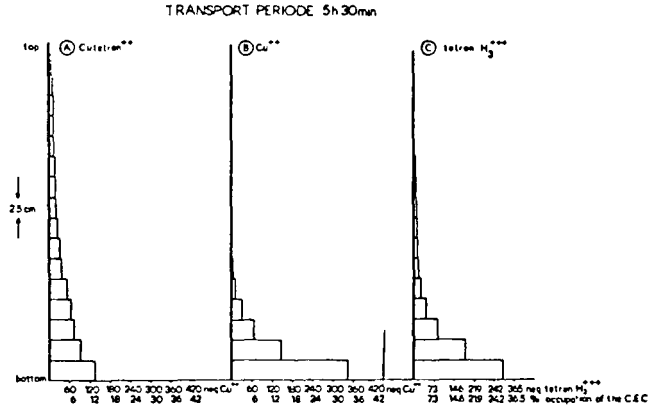

Fig. 3. Distribution of Cu or C-tetren in a papyrus stem after 330 minutes of absorption. The solutions were:
A: Cu-Tetren 10^{-6} mole/l, $Ca(NO_3)_2$ 5.10^{-4} mole/l
B: $Cu(NO_3)_2$ 10^{-6} mole/l, $Ca(NO_3)_2$ 5.10^{-4} mole/l
C: tetren $H3^{3+}$ 10^{-6} mole/l, $Ca(NO_3)_2$ 5.10^{-4} mole/l

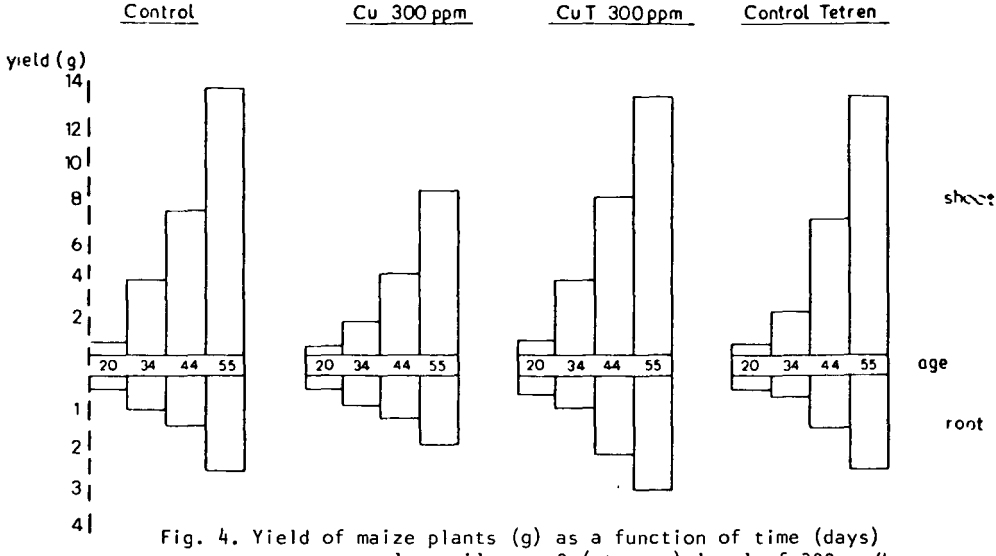

Fig. 4. Yield of maize plants (g) as a function of time (days) grown on a clay soil at a Cu(-tetren) level of 300 mg/kg. The "control tetren" treatment was amended with the same amount of tetren as the Cu T treatment.

Publications

Smeulders, F.G.H. *In situ* immobilization of transition metal ions in soils with tetraethylenepentamine. Doctoral thesis, K.U.L., Leuven, Belgium (1980).

Smeulders, F.G.H., J. Sinnaeve and A. Cremers. *In situ* immobilization of heavy metals in soils. Proc. 8th Int. Coll. on Plant Analysis and Fertilizer Problems, Auckland, New Zealand, 1978 (Ed. by DSIR, New Zealand).

Resultaten van het project No. 7

Hoofd van het team en wetenschappelijke medewerkers:

G. Desmet, J. Sinnaeve, S.C. van de Geijn, A. Ringoet

Titel van het project:

Root and foliar uptake of radioactive contaminants by crops; their transport and redistribution in different plant organs (e.g. edible parts, reproductive organs).

Beschrijving van de resultaten:

The aim of this project has been the determination of transfer coefficients and (re)distribution of some radiocontaminants in plants. The subject has been assessed in two different ways. Firstly experiments have been performed with plants growing on nutrient solutions to deal with problems of growth mechanisms which often provoke conflicting interpretations of transfer functions, and secondly experiments have been performed in soils in order to obtain insight into the order of magnitude of the transfer coefficients.

The nutrient solution experiments utilized both spinach (*Spinacea oleracea* L. cv. Verbeterd Breedblad) and tomato plants (*Lycopersicon esculentum* Mill. cv. Moneymaker). The data on spinach relating age and transfer are presented in table 1. The ^{54}Mn and ^{60}Co transfers have been studied at one fixed stable isotope concentration and the ^{65}Zn transfer at three stable isotope concentrations. Large differences among the three radioisotopes' transfers were observed with a clear dependence on the age of the plants.
Moreover in the ^{65}Zn experiments a dependence on the stable isotope concentration was established. In tomato plants the roots and even more the fruits were the most important sites of accumulation of ^{65}Zn (table 2).
For all plant organs, the amount of ^{65}Zn accumulated varied considerably with the degree of maturation at harvest time. All these data are firm evidence of the existence of predictable transfer functions instead of randomly occurring transfer factors.

A comparison between the amount of Zn "supplied" with the water taken up and the actual Zn consumption of a spinach plant rules out the opportunity to utilize water-uptake data for transfer calculations of radiocontaminants in forecasting models (table 3).

The mobility in spinach leaves of ^{65}Zn and ^{60}Co has also been studied by investigating the redistribution of ^{65}Zn after a short period of contamination, and by measuring the time necessary for ^{65}Zn and ^{60}Co to exchange with stable Zn and Co in mature non-growing leaves. The latter method will be published in detail elsewhere. Both approaches showed a strong binding of zinc, with almost no redistribution and with very slow isotopic exchange rates. The ^{60}Co in contrast showed a very rapid exchange rate. In order to study the distribution of ^{65}Zn within tomato plants a natural sandy-loam soil column was contaminated over its complete length at different times after the appearance of the first flowers. The ^{65}Zn content of the lower part of the stem was higher than of the upper part and this difference sharply increased with a shortening of the contamination period (a factor 4 for a 40 days contamination period compared to a factor 15 for an 18 days contamination period). However, the ^{65}Zn distribution, being mainly in the seeds and the peel was hardly influenced by time of the contamination.

Prior to the determination of transfer coefficients of ^{65}Zn from a well defined clay soil to the spinach, several introductory studies of some important parameters have been done. These included the growth rate of spinach at various soil humidities, the rate of extraction of stable Zn and the total availability of the native Zn and of the artificially supplied ^{65}Zn. In this soil about 40% of the ^{65}Zn appeared to be tightly adsorbed, the other 60% being extractable by the commonly used soil extractant DTPA.
The transfer of ^{65}Zn after both a top and a bottom layer contamination of the clay soil has been investigated in pot experiments. The top layer contamination caused rather small contaminations of the leaves (table 4) because of the very small depth migration of the ^{65}Zn (also verified by soil column scanning experiments) and the fact that the roots were rapidly penetrating the soil. This penetration was verified in the bottom layer contamination experiment.
Comparative transfer studies of different radionuclides were performed with several plant species growing on a sandy-loam soil. The plants used were tomato, maize (*Zea mays* L. cv. spec.), bean (*Phaseolus vulgaris* L. cv. Witte Krombek), barley (*Hordeum vulgare* L. cv. Piccolo), lupine (*Lupinus luteus* L. cv. Barpine), wheat (*Triticum sativum* L. cv. Tundra), clover (*Trifolium repens* L. cv. Retor), spinach and ryegrass (*Lolium perenne* L. cv. Pelo). The tomato plants were grown in a uniformly labelled soil whereas for the other cultures only the upper 7 cm of the soil were contaminated. The results are presented in table 5. The transfer coefficients of the divalent cations with the exception of iron are rather high, being on average higher than of the monovalent cation Cs. Calculations of transfer coefficients are hazardous hitherto in top layer contamination since no certainty exists about the radio-contaminants' concentration at the sites where it is picked up by the individual roots. They depend moreover on soil water content. Further research is therefor urgently needed to assess these problems.

Table 1 - The dependence of the transfer coefficient (TC) upon age or concentration. TC = cpm per kg fresh weight/cpm per l nutrient solution

	Age of plants days	Transfer coefficients		
		cotyledons	first leaves	second leaves
Stable Zn concentration mole/l soln. $1,76.10^{-7}$	6	108	176	
	10	46	164	238
	13	56	80	167
	17	49	58	118
	22	47	54	127
$4,40\ 10^{-6}$	6	23	40	
	10	33	42	55
	13	32	34	36
	17	29	29	24
	22	23	22	22
$2,64\ 10^{-5}$	6	6,7	10	
	10	8,8	10	8,6
	13	10	11	8,7
	17	8,8	9,2	6,5
	22	10	9,2	6,9
Stable Mn concentration $1,16\ 10^{-5}$	9	13	12	6
	11	19	17	13
	14	21	18	14
	18	34	24	16
	21	57	24	15
Stable Co concentration 1.10^{-8}	1	1,1		
	8	2,3	5,1	
	13	2,3	3,4	5,2
	21	2,6	1,6	1,5
	27	2,1	1,4	1,2

Table 2 - ^{65}Zn content in plant organs at different ages

Age or part of plant organ	^{65}Zn content, expressed in the dry matter					
	leaves		petioles		stem	
	µCi/g	MBq/kg	µCi/g	MBq/kg	µCi/g	MBq/kg
Old	0,59	22	1,55	57		
Medium	1,13	42	2,58	95		
Young	2,23	83	2,93	108		
Base					0,43	16
Top					1,36	50

Table 3 - Comparison between the amount of zinc present in the daily water consumption by a spinach plant at different zinc concentrations and the total amount of zinc accumulated in one day. The mean and standard deviation are presented, n = 4 with 9 plants on a holder at 5 days, and 3 per harvest at 17 days. - means shortage; + means surplus.

Parameter	Concentration of Zn		
	$1,76 \times 10^{-7}$ mole/l	$4,40 \times 10^{-6}$ mole/l	$2,64 \times 10^{-5}$ mole/l
Age of plant: 5 days			
Zinc in daily water consumption, nmole	0,07± 0,00	1,77± 0,02	10,4± 0,48
Zinc accumulated in one day, nmole	4,15± 1,98	9,15± 3,44	20,2± 8,6
Difference, nmole	-4,08± 1,98	-7,38± 3,44	-9,8± 8,6
%	-98	-81	-48,5
Age of plant: 17 days			
Zinc in daily water consumption, nmole	1,67± 0,14	54,6 ± 3,4	372 ± 31,4
Zinc accumulated in one day, nmole	57 ±36,4	147 ±67,5	350 ±253
Difference, nmole	-55,3 ±36,4	-92,4 ±67,6	+22 ±255
%	-97	-63	+6

Table 4 - Depth migration of ^{65}Zn after a top layer contamination and transfer coefficients (counts in 5 min per g dry weight of plant / counts in 5 min per g dry soil top layer) in spinach.

Soil water content %	Depth migration layers cm	Counts in 5 min per g dry soil		Plant part	Transfer coefficient
		total	extract		
15	0 - 2	23740	15850	cotyledons	0,50 ± 0,18
	2 - 4	628	407	first leaves	0,60 ± 0,04
	4 - 6	55	34	second leaves	0,57 ± 0,16
	6 - 8	14	5		
25	0 - 2	21510	12350	cotyledons	0,11 ± 0,02
	2 - 4	289	199	first leaves	0,15 ± 0,11
	4 - 6	210	137	second leaves	0,14 ± 0,09
	6 - 8	50	32		

Table 5 - Mean transfer coefficients of the shoot (activity concentration of dry matter divided by activity concentration of dry soil) and standard deviations (four replicates) for different radionuclides and different plant species.

Plant species	Transfer coefficient of the shoot					
	^{60}Co	^{65}Zn	^{59}Fe	^{54}Mn	^{90}Sr	^{134}Cs
tomato*	0,03±0,02	2,46±1,15	-	2,60±1,04	-	2,22±0,24
bean	3,29±1,10	0,52±0,24	0,06**	1,33±0,68	1,47+0,28	0,05±0,03
barley	0,64±0,08	0,30±0,18	0,09±0,03	0,38±0,18	0,38±0,14	0,11±0,08
maize	0,17±0,09	0,41±0,09	0,03**	0,70±0,41	0,30±0,14	0,05±0,02
lupine	6,66±3,13	0,42±0,34	0,10±0,08	1,15±1,21	1,32±0,93	0,15±0,14
wheat	0,41±0,11	0,28±0,04	0,06±0,02	0,56±0,13	0,27±0,07	0,11±0,04
clover	2,76±1,68	0,53±0,14	0,09±0,02	0,76±0,20	0,94±0,17	0,14±0,06
spinach	2,97±0,95	2,00±0,72	0,04±0,01	2,70±0,56	0,64±0,23	0,04±0,01
ryegrass	0,64±0,44	0,38±0,08	0,07**	0,44±0,02	0,42±0,13	0,07±0,01

* homogeneous contamination of the soil

** mean value of two replicates

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Sinnaeve, J., F. van Dorp. Mixtures of synthetic ion exchangers as an artificial soil for plant nutrition studies. Proc. 8th Int. Coll. on Plant Analysis and Fertilizer problems, Auckland, New Zealand, 1978.

Sinnaeve, J. A growth chamber for the integrated study of the plant-soil-atmosphere interactions. Application to the influence of soil temperature on the mineral composition and development of plants. Proc. 8th Int. Coll. on Plant Analysis and Fertilizer Problems, Auckland, New Zealand, 1978.

Sinnaeve, J. Technical description of a growth chamber for the study of soil-plant-atmosphere interactions. J. Exp. Bot. (in press).

Resultaten van het project No. 8

Hoofd van het team en wetenschappelijke medewerkers:

J. Sinnaeve, S.C. van de Geijn, M.J. Frissel, N. van der Klugt

Titel van het project:

Behaviour of radionuclides in biological and non-biological processes at very low concentrations.

Beschrijving van de resultaten:

The aim of the project was to investigate an observed aberrant behaviour of two isotopes of the same element at very low concentrations. These results were reported in 1974. The data discussed here were obtained under experimental conditions identical throughout the study. However, new counting equipment, i.e. a germanium-lithium drifted detector with a resolution of 2,1 keV at the 1,31 MeV photopeak of ^{60}Co, has been used. For all counting results, the peak areas of the identified photopeaks (corresponding with the γ-photons with 605 and 796 keV for ^{134}Cs and 662 keV for ^{137}Cs) were computed by Gaussian fitting and base-line correction using a modified "GASPAN" fitting procedure. After correction for radioactive decay, the Z-value of the sample, i.e. the ratio of the activity concentrations of ^{134}Cs and ^{137}Cs in the plant material divided by that ratio in the reference sample, mostly the nutrient solution, was computed.

A. Biological aspects.

Plant uptake experiments were carried out with intact tomato plants (*Lycopersicon exculentum* Mill., cv. Marette VF). During the experimental period, the nutrient solution contained stable caesium (mostly between 0,3 and 3 mg per liter), and ^{134}Cs and ^{137}Cs at the indicated activity ratio. The results of the key experiment (reported in 1974) clearly indicated aberrant Z-values for the root samples at different activity ratios. After four years of storage of the samples, they were recounted with the new counting equipment. An extract of the results is given in table 1. The second counting confirmed the anomalies reported previously and this led to several experiments in order to answer specific questions.

1. Is an absorption step involved which is linked to metabolism? Absorption experiments were carried out with nutrient solutions labelled with 1,9 kBq/l (0,05 µCi/l) ^{134}Cs and 90 kBq/l (2,5 µCi/l) ^{137}Cs and kept at 22, 12 and 2 °C respectively during the experimental period. To investigate whether a time dependent phenomenon was involved, two plants of each treatment were harvested after 150, 300, 450 and 600 minutes. The results are given in table 2. As no influence of time was observed, the mean values of the eight replicates per treatment are given. Taking into account the rather large standard deviations (can be up to 8%), it can be concluded that none of the Z-values is significantly different from 1.

2. Are different physico-chemical forms of the isotopes involved? Plants were grown on a mixture of a weak acid and a weak base ion exchanger saturated with the nutrient elements. 11, 7, 5, 3 and 1 day before harvest of the plants (age 30 days), 20 ml of a solution containing 24 mg stable caesium per liter, 1,9 kBq (0,05 µCi) ^{134}Cs and 900 kBq (25 µCi) ^{137}Cs, were applied on top of the substrate. The Z-values were computed using as a reference sample

Table 1 - Mean Z-values and their standard deviations (five replicates) for root samples using different labelling procedures

Counting date	^{134}Cs photo-peak keV	Mean Z-values ± standard deviation			
		$^{134}Cs/^{137}Cs$ activity concentrations in kBq/l (µCi/l)			
		1,85/92,5 (0,05/2,5)	18,5/185 (0,5/5,0)	185/18,5 (5,0/0,5)	92,5/1,85 (2,5/0,05)
Dec. 1973	796	2,11 ± 0,05	1,00 ± 0,01	0,78 ± 0,02	0,35 ± 0,04
Dec. 1977	796	1,92 ± 0,09	1,02 ± 0,03	0,77 ± 0,05	0,36 ± 0,10
Dec. 1977	605	1,73 ± 0,11	1,02 ± 0,03	0,78 ± 0,06	0,36 ± 0,10

Table 2 - Mean Z-values and their standard deviations for roots (R), stems (S) and leaves (L) using different photopeaks of ^{134}Cs

Temperature	Plant organ	^{134}Cs photopeaks used		
		605 keV	796 keV	605 + 796 keV
22 °C	R	1,00 ± 0,02	1,02 ± 0,04	1,01 ± 0,02
	S	0,99 ± 0,01	1,00 ± 0,01	0,99 ± 0,01
	L	1,00 ± 0,01	1,00 ± 0,02	1,00 ± 0,01
12 °C	R	0,98 ± 0,03	1,00 ± 0,03	0,99 ± 0,03
	S	0,99 ± 0,03	1,01 ± 0,03	1,00 ± 0,03
	L	1,02 ± 0,01	1,10 ± 0,05	1,05 ± 0,02
2 °C	R	1,00 ± 0,03	1,01 ± 0,02	1,02 + 0,02
	S	1,00 ± 0,02	1,00 ± 0,06	1,01 ± 0,04

the equilibrium solution of the substrate and the substrate itself. Given the extreme activity ratio used, no significantly deviating Z-values were found.

3. Are biophysical exchange phenomena involved?
Exchange chromatography using biological exchange columns (cut papyrus stem - *Cyperus papyrus* L. spec. - with a cation exchange capacity of about $8,10^{-5}$ eq/m and a length of about 90 cm) was carried out to check whether different isotopic behaviour at the solution-membrane interphase might occur. Untreated, protonated and potassium charged "exchange columns" were used. In total, some fifteen experiments with different acitivty ratios were carried out but no aberrant behaviour could be observed.

B. Non-biological aspects.

1. Exchange chromatography
To test whether aberrant behaviour at the solution-root interphase might occur, exchange chromatography experiments with glass pearls, having a low cation exchange capacity, were carried out. Prior to exchange with a solution containing 1,9 kBq/l (0,05 µCi/l) ^{134}Cs and 900 kBq/l (25 µCi/l) ^{137}Cs - with and without 25 mg/l stable

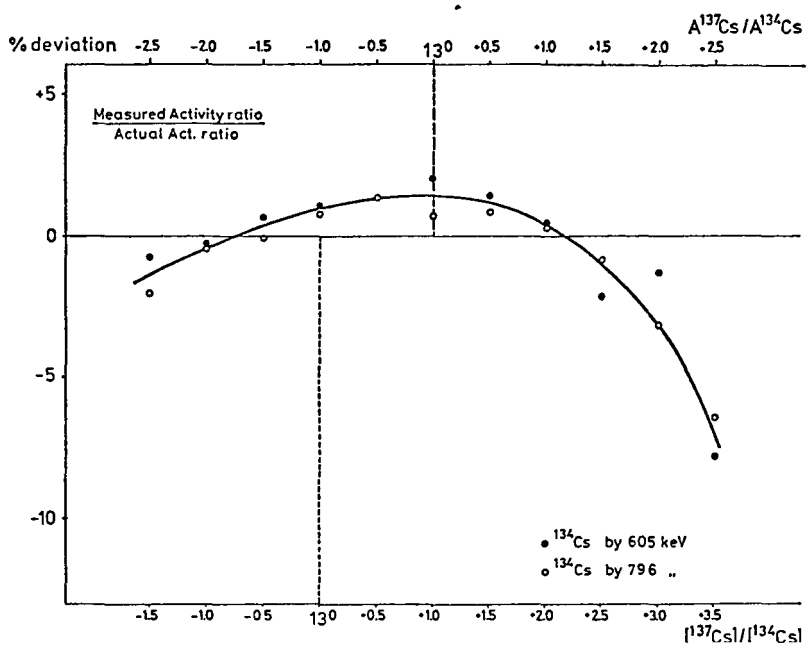

Fig. 1. Deviations of activity ratios.

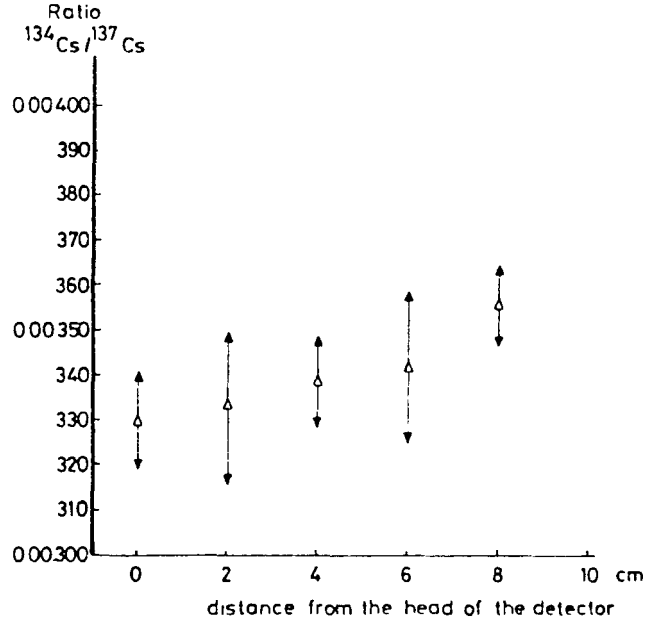

Fig. 2. $^{134}Cs/^{137}Cs$ ratio of a sample (based on the 795 keV photopeak of ^{134}Cs) as a function of counting rate (decreasing with increasing distance).

caesium -, the exchange sites were brought in the H^+-form. The Z-values of all percolated fractions were normal

2. Counting equipment
The response of the counting equipment towards various $^{134}Cs/^{137}Cs$ activity and concentration ratios in the absence of any physical, chemical or biological effects has been tested. The ratio of the activity concentrations A of both isotopes varied in geometric sequence ranging from $13^{-2,5}$ to $13^{2,5}$ ($1,6 \times 10^{-3}$ to 610) by exponential increments of 0,5. The logarithmic base 13 being equal to the ratio of the decay constants, the activity series can be superposed to a mass concentration ratio ranging from $13^{-1,5}$ to $13^{3,5}$ (0,02 to 7900). The deviations of the measured activity ratios from the prepared ones, expressed in percentage of the theoretical values, are represented in figure 1. A systematic effect exists which reduces the $^{137}Cs/^{134}Cs$ ratio when the activity ratio is greater than 50.
In collaboration with Drs. Myttenaere and Ronneau (U.C.L., Louvain la Neuve, Belgium) and Dr. Guillot (CEC, Brussels, Belgium) the influence of counting rate on the ratio of the activity concentrations of ^{134}Cs and ^{137}Cs was checked. The results given in figure 2 illustrate a slight systematic shift over the entire range of counting rates of the samples. These deviations cannot explain the reported anomalies.

C. Conclusion

In the light of the reported data, it may be concluded that systematic differences in biological and non-biological processes of isotopes and stable elements at extreme concentration ratios are absent. The anomalies observed before must therefore be attributed to an artefact - most probably a difference in physico-chemical form - and this conclusion stresses the necessity of careful use and interpretation of experiments on and with radiotracers.

Contractant de la Commission : Institut d'Hygiène et d'Epidémiologie
Ministère de la Santé Publique, Bruxelles.

N° du contrat : 258-77-7 BIO B.

Chef du groupe de recherche : G. CANTILLON

Thème général du contrat : Etude de l'impact des rejets provenant des centrales nucléaires de type PWR sur la biocénose dulcicole.

Titre du projet n° 1 : Etude de l'impact des rejets provenant des centrales nucléaires de type PWR sur la biocénose dulcicole.

Chef du projet et collaborateurs scientifiques : G. CANTILLON, J.P. DESCY, H. DE CLERCQ, A. EMPAIN, M. GENIN, R. KIRCHMANN, M. MEURICE, G. VERFAILLIE

Les rejets d'effluents provenant des centrales nucléaires peuvent modifier les caractéristiques thermiques, radioactives et chimiques du cours d'eau récepteur et donc agir sur les êtres vivant dans ce milieu. Le site de la centrale de Tihange se prête relativement bien à une étude comparée des êtres vivants de la Meuse, en amont et en aval du point de rejet de l'effluent considéré, afin de déceler les altérations éventuelles subies par l'écosystème. Afin de mieux préciser l'impact réel et global des rejets sur les organismes aquatiques, le programme prévoit l'installation de supports et cages contenant divers types d'organismes (producteurs primaires, consommateurs primaires et secondaires, décomposeurs) dans le canal d'amenée de l'eau de refroidissement de la centrale ainsi que dans le canal de rejet des eaux usées. Cette étude faite ainsi sur les rejets de la centrale PWR de Tihange I peut servir de modèle pour les eaux douces d'autres régions européennes ayant des caractéristiques comparables et peut s'appliquer à la plupart des installations nucléaires actuelles ou prévues dans la Communauté. En outre, des expériences en laboratoire visant à étudier le comportement de végétaux verts cultivés dans les effluents produits par la centrale.

1. Impact des rejets thermiques.

Le fonctionnement de la centrale PWR de Tihange I (870 MWe) provoque une augmentation moyenne de la température de l'eau de la Meuse de 2 à 3° C; cette différence amont-aval s'accentue en été et atteint 5 à 6° C pendant la période d'étiage. Jusqu'à présent, il ressort des analyses que cet échauffement n'a pas eu de conséquences importantes en aval immédiat de la centrale au niveau de l'oxygène dissous et des paramètres qui lui sont liés mais elles se manifestent plus en aval, où le cumul d'autres rejets thermiques (centrale électrique conventionnelle et industries) crée des situations critiques. Les études in situ des peuplements de diatomées et de bryophytes aquatiques montrent une évolution qui est bien corrélée avec les résultats des analyses physico-chimiques : aucune dégradation n'est observée immédiatement en aval mais elle se manifeste au-delà; de plus, l'augmentation de température et la turbidité importante de l'eau déplacent l'équilibre photosynthèse/respiration vers un déficit de synthèse et donc aussi vers un déficit de libération d'oxygène. Par contre, l'influence du réchauffement de l'eau ne semble avoir aucun effet délectable du point de vue qualitatif sur la faune des invertébrés et sur le peuplement piscicole de la Meuse. Cependant, la croissance des macro-invertébrés a été modifiée mais non celle des poissons (gardons); chez ces derniers, l'étude du cyle de reproduction en amont et en aval des rejets de la centrale nucléaire a permis de constater des différences entre les populations amont et aval. En ce qui concerne le phytoplancton dont l'étude a commencé en 1979 seulement, les résultats ne mettent pas en évidence un effet marqué de la centrale sur les teneurs en chlorophylle et en phaeophytines, bien que souvent les teneurs observées en chlorophylle sont lègèrement plus élevées (15 %) en aval; de même, une légère différence (10 %) a été observée dans l'activité photosynthétique.

2. Impact des rejets radioactifs.

A l'inverse des mollusques, les poissons concentrent par ordre décroissant le ^{137}Cs, le ^{60}Co et le ^{54}Mn dans leurs tissus. A noter que le ^{137}Cs se répartit sélectivement suivant les différents organes, la rate et les gonades accusant les teneurs les plus élevées. Parmi des différentes espèces de poissons étudiées, les perches, consommatries secondaires et même tertiaires, présentent des teneurs en ^{137}Cs les plus élevées. L'homme constitue le maillon final de la chaîne alimentaire; en effet, la pêche récréative est développée sur le secteur étudié. Il a été constaté que la forte diminution des activités volumiques en ^{137}Cs ajoutées dans la rivière (1978 et 1977 par rapport à 1976) s'est traduite par une diminution des activités mesurées dans les poissons, confirmant la qualité de ces derniers en tant que bio-indicateurs.

Par ailleurs, la culture d'algues (Scenedesmus obliquus) en laboratoire sur des milieux contenant des effluents liquides de Tihange I a permis de conclure que la disponibilité biologique des paires de radioéléments $^{58}Co/^{60}Co$ et ^{134}Cs était similaire. Ces cultures ont d'autre part mis en évidence, par bioaccumulation, la présence de certains radionucléides, non détectables par la mesure directe.

3. Impact des rejets chimiques.

Le biocide utilisé par la centrale Tihange I, pour empêcher le développement des algues dans les tours de réfrigération, étant l'hypochlorite de soude, les expériences en laboratoire ont porté sur ce produit. Les courbes "Dose-effets" ainsi que le taux de "récupération" ont été établies pour l'algue Scenedesmus obliquus. Par ailleurs, les travaux de laboratoire portant sur la physiologie des bryophytes ont montré que l'impact d'une chloration (40 minutes) ne se décèle sur la capacité photosynthétique que lorsque les concentrations en chlore libre sont de l'ordre du mg/l. Lors d'une chloration réelle à la centrale, la capacité photosynthétique d'une culture expérimentale a été diminuée de 15 à 20 % après un temps de récupération de 24 h. De même, une réduction progressive de la biomasse algale a été constatée à partir de juillet dans le canal de rejet alors qu'elle se maintient à une valeur normale en amont; cette dégradation progressive paraît surtout liée à la période des chlorations hebdomadaires des eaux de rejets de la centrale.

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RAPPORTS :

CEN/SCK-IHE, 1977 : Etude de l'impact du rejet provenant de Centrales Nucléaires de type PWR sur les écosystèmes d'eau douce et marine.

	IHE	Bruxelles	Vol.	I	période	1976-1977
1978	id.	"	Vol.	II	"	1 semestre 1977
1980	id.	"	Vol.	III	"	juillet 77 à juin 78
1981	id.	"	Vol.	IV	"	juillet 78 à déc. 79

(en préparation)

PUBLICATIONS :

MICHA et MEURISSE-GENIN 1977 : Impact des Centrales Nucléaires sur les écosystèmes dulcicoles : Les Naturalistes belges 59,149-158.

Id. 1977 : Contamination radioactive de la faune aquatique dulcicole. Annales Ass. belge de radioprotection 2/3 217-237.

Id. 1980 : Impact des rejets radioactifs provenant d'une Centrale Nucléaire du type PWR sur les poissons de la Meuse - Revue des Questions Scientifiques 151/2 221-234.

Contractor : Facultés Universitaires Notre-Dame de la Paix (Namur)

Results of contract n° : 265-79-3 BIO B

Project Leader : Prof. Dr. J.-C. Micha, A. Detollenaere

Title of project : Effects of Thermal Pollution of a Nuclear Power Plant on Aquatic Organisms (Macroinvertebrates and Fishes) of the River Meuse.

The object of this research was to determine the thermal impact of a nuclear power plant (PWR type) Tihange I (872 MWe) on macroinvertebrates and fish living in the river Meuse.

Temperatures recorded

The water temperature of the Meuse was continuously recorded upstream and dowstream of the nuclear power plant. At the recording station downstream, effluent and river water are fully mixed. The change in the average daily temperatures of the water which was calculated with reference to the data taken at midnight, 6 a.m., midday and 6 p.m., revealed a seasonal thermal cycle which ranged from 0° to 21°C upstream and from 0° to 24°C downstream (1979-1980). Thermal discharges of the nuclear power plant fluctuated from 70 Mcal/sec to 400 Mcal/sec according to temperature and flow conditions and resulted a perceptible heating of about 2-3°C. In general, the thermal differences between the two parts of the river are very small during the winter (end of November to beginning of March), increase in Spring and reach 3-4°C in Summer and in Autumn. The maxima-minima range of daily temperatures is generally more important downstream (0,8°C instead of 0,3°C upstream in November 1979).

Effects on macroinvertebrates

So far, about 44 taxa of macroinvertebrates have been identified from both areas. With few exceptions, Oligochetae (2), Triclads (6), Hirudinae (3), Molluscs (8), Crustacea (5), Insect larvae (20) have been recorded upstream as well as downstream. However, Triclad Dugesia tigrina was regularly found in greater abundance downstream.

In 1978, investigations on aquatic invertebrates populations showed that molluscan bivalve Dreissena polymorpha and shrimp Atyaephyra desmaresti which were living at the lower sampling site, seemed to grow

at a higher rate during Spring. Furthermore, artificial water heating appeared to extend the growing season beyond October, this is true for both species. In 1979-1980 unfortunately, Dreissena polymorpha and Atyaephyra desmaresti were seldomly found along the river banks, and were especially very rare at the upstream of the nuclear power plant. It's the reason why no comparison was realized throughout that period. Nevertheless, data available downstream, for mussels as well as for shrimps, allowed us to confirm first results on growth and reproduction pattern of these two species. Moreover, inventories on feeding and fecundity of Atyaephyra desmaresti were carried out. In other respects, preliminary experiments concerning Atyaephyra desmaresti suggest a lethal temperature (TL 50/24 h) of 25°C, the acclimatation temperature being 15°C.

Effects on fish

Thermal elevation of water seems to have no noticeable effect from a qualitative point of view on the fish populations of the Meuse. In fact, in the monthly catches (March 1977 to October 1980) at upstream as well as downstream, the presence of 23 species belonging to 8 families (13 Cyprinidae, 1 Percidae, 1 Esocidae, 1 Anguillidae, 1 Centrarchidae, 1 Gasterosteidae, 1 Salmonidae, 1 Cobitidae) was noted. Among these species, roach Rutilus rutilus L. (Cyprinidae) is found in particular abundance and is the most easily caught with our equipment (mesh nets and electrofishing). It is the reason why more emphasis has been placed on growth, feeding and reproduction of this species.

The length-frequency distributions were obtained throughout the year from 200 young roach samples (< 17 cm) collected along the upper river banks and revealed a seasonal growth pattern : average length increase was minimal between October and June and remained relatively slow during summer months. On the other hand, the weight-length relationships, calculated for 1979 and 1980, separately for young roach (< 17 cm) and females (> 19 cm) caught in both areas, revealed no significant differences between coefficients b of roach populations living upstream and downstream of the nuclear power plant at Tihange.

Contents of the alimentary canal of 656 roach, collected throughout 1976 and 1979-1980 were examined. Inventories showed that :

- Filamentous algae, diatoms, detritus, molluscs and arthropodes provided the bulk of roach diet. Slight upstream-downstream differences appear in food composition, particularly in "1976" samples : higher frequency of molluscs and algae downstream,and detritus upstream.

- There is an increase in the frequency of empty "stomachs" during the winter months. This seasonal change is less pronounced at the lower site.

Examination of the reproduction of roach, based on the development of gonado-somatic relationship (RGS) reflected, in the female and male roach, the seasonal cycle of the temperature and of the photoperiod : spawning occured during the latter half of May when temperature reached 14,5°-15°C, and was followed by a quiescent period of male and female gonad activity until September when elaboration of gonad tissue restarted. Ovary weight steadily increased during the winter and peaked in April-May. At the end of May, beginning of June, a sudden fall in the roach RGS was noted which coïncided with the end of spawning period. The thermal discharges of the nuclear power station slightly influence this development. By December, the average RGS of the females is already higher downstream. This difference is maintained throughout the winter and generally increases till May just before reproduction. Explanation for this phenomenon has been found by making investigations on fecundity of 225 females caught in April-May 1979 and from January to May 1980. Higher RGS observed downstream seems to correspond more to an earlier development and to a greater proportion of large ripening eggs that to a greater number of eggs. In May 1979 and 1980, artificial spawning "areas" were set up at various places along the river banks and were checked daily. Observations revealed that :

- The eggs just laid down finally by downstream and upstream females had the same diameter (1,70 mm).

- In opposite to 1977 when breeding period occured two weeks earlier downstream, reproduction took place around mid-May at the upper site as well as the lower's.

- Close to reproduction period, thermal differences between the two sampling sites were very small in 1979 ($\leqslant$ 2°C) and didn't exist in 1980 (as a result of the non-functioning of the nuclear power station).

In conclusion, the installation of a first reactor (872 MWe) of the nuclear power plant at Tihange causes, especially in Autumn, a perceptible heating of the river Meuse. So far, this slight modification in the thermal rate of the river does not greatly affect macroinvertebrates and fish populations living in the river Meuse. The few differences observed between the upper and lower sampling sites are comparable with natural changes between two isolated populations.

This work has attempted to collect a maximum of informations and to put the emphazis on growth, feeding and reproduction cycles of macroinvertebrates and fish living in the river. The interest of this study was to draw up a balance of the situation in Meuse at the present time since no work has been done before, with regard to this problem. It is the reason why such a search must be carried on as the construction of new reactors is in process and when they'll become into operation (1981 and 1983) the hydro and thermal balance of the river Meuse will probably be disturbed at an increasing rate.

Publications

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- Detollenare, A. et Micha, J.-C., 1980. Effets des rejets thermiques d'une centrale nucléaire sur les poissons de la Meuse. Revue des Questions Scientifiques, 151 (1).

Contractant of the Commission: Delta Institute for Hydrobiological Research
Royal Netherlands Academy of Sciences
Vierstraat 28
4401 EA YERSEKE
The Netherlands

Contract number: 268-79-1-BION
Head of Research team: Dr. E.K. Duursma (coordinator)
General Subject: Plutonium in the Rhine-Meuse-Scheldt delta; analysis of estuarine sediment, salt-marsh soil and vegetation samples.

The Rhine-Meuse-Scheldt estuaries might be envisaged as sinks for many substances which are transported by the rivers Rhine, Meuse and Scheldt to the sea. Identically through tidal movements contaminants transported by the North Sea might arrive in these estuaries.

A research was made to determine ^{238}, ^{239} and ^{240}Pu concentrations in sediment, particulate matter, salt-marsh plants and lichens from various locations of the Delta area (Fig. 1). The objectives were to trace the sources of plutonium for this area, either being fallout, up-river nuclear installations or the reprocessing plant in La Hague (transport along the coast).

Not all 1980-samples have been analysed yet,which are in particular the particulate matter samples taken outside the Western Scheldt area and the lichen samples.

Sub-Project 1. Delta Institute for Hydrobiological Research
Head research team: Dr. E.K. Duursma
General subject: Collection and preparation of samples, and additional analysis by chemical methods.

For 1980 additional salt-marsh sediments, particulate matter and old salt-marsh sediment samples from our stock have been analysed on humidity, salinity, $CaCO_3$, POC (particulate organic carbon), total nitrogen, potassium (K), clay, total α-radioactivity and pH.

The total α-radioactivity reflects all natural and possible artificial

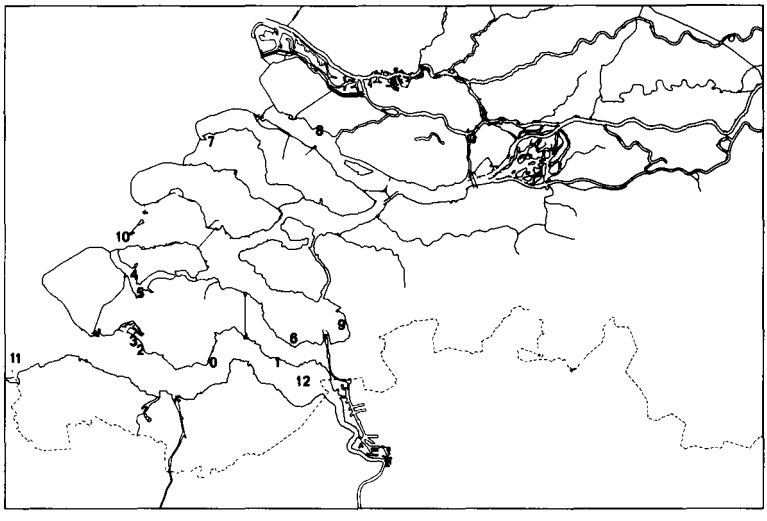


Fig. 1. Rhine-Meuse-Scheldt estuaries.
Sampling stations: (0) Ellewoutsdijk, (1) Waarde, (2) Borssele, Nuclear Power Station, (3) Kaloot), (4) Spieringschor, (5) Noordsloe, (6) Stroodorpepolder, (7) Springersgors, (8) Zuidland, (9) Salt marsh Bergen op Zoom, (10) Mouth Eastern Scheldt, (11) Wielingen, (12) Nauw van Bath.

Table I Sediment and suspended matter parameters per dry weight For sampling stations see Fig 1

	samples	humidity/ wet weight %	salinity- interst water ‰	$CaCO_3$ %	POC %	tot N %	K meq/100 g	Clay %	α radioact $pCi\ g^{-1}$	pH-KCl	α rad/clay $pCi\ g^{-1}$
Salt-marsh sediment											
Waarde	20-40 cm	6'79	47 3	18 5	14 6	3 63	0 18	1 73	35 4	5 5	7 74	15 5
(1)	40-60 cm	6'79	50 0	21 1	13 8	3 86	0 19	1 76	35 8	5 4	7 65	15 3
	60-80 cm	6'79	47 0	19 3	14 3	3 58	0 19	1 89	36 9	7 4	7 64	20 1
	80-100cm	6'79	40 9	18 5	15 8	3 97	0 17	1 72	37 2	4 3	7 60	11 6
Borssele	0-10 cm	3'80	17 2	22 9	2 62	0 32	0 01	1 34	0 4	3 3	8 65	825 -
(2)	10-20 cm	3'80	19 0	17 5	7 75	0 76	0 28	1 26	1 7	3 2	8 12	188 3
Old salt-marsh sediments (0-10 cm)											
(3)	Kaloot	5'62		21 9	8 9	1 16	0 11	1 23	18 9	4 4		23 3
(4)	Spieringschor	7'62		3 80	13 0	2 96	0 29	2 23	56 3	5 2		9 2
(5)	Noordsloe	8'62		0 30	7 5	1 86	0 19	1 82	36 6	4 2		11 5
(6)	Stroodorpepolder	3'65		20 3	3 4	1 24	0 06	1 37	10 7	5 1		47 7
	idem	7'68		26 0	5 0	1 42	0 07	1 39	12 4	0 7		5 6
(7)	Springersgors	8'62		30 9	7 0	4 23	0 31	2 82	30 1	n d		n d
	idem	7'68		17 1	9 2	6 91	0 46	2 41	32 5	3 5		10 8
	idem	7'7		29 6	12 1	7 67	0 45	4 45	58 9	4 5		7 6
	idem	10'72		2 26	12 2	5 39	0 50	3 32	59 6	4 2		7 0
(8)	Zuidland	8'61		3 2	12 4	2 15	0 13	0 65	15 0	4 7		31 3
(9)	Salt marsh Bergen op Zoom	3'64		26 4	1 6	0 10	0 02	0 11	1 5	1 8		83 3
	idem	6'75		15 1	5 1	3 53	0 30	2 90	18 0	4 8		26 7
	idem	5'78		24 9	5 2	3 73	0 26	2 52	26 6	4 7		17 7
Suspended matter											
(10)	Mouth Eastern Scheldt	6'80	55 7	30 4	7 86	4 06	0 51		11 3	3 9	9 09	17 7
(11)	Wielingen (high-tide)	7'80	47 3	26 1	27 5	4 29	0 31		39 7	3 7	7 93	9 1
	idem (low-tide)	7'80	50 7	20 7	18 5	4 85	0 38		41 0	4 2	7 94	10 2
(12)	Nauw van Bath	9'80	53 3	9 75	1 51	6 15	0 49		66 3	10 5	7 39	15 8

Fig. 2. Concentration relationship between ^{137}Cs and $^{239,\ 240}Pu$ of estuarine sediments and suspended material for three estuaries: Rhine-Scheldt delta (this study), the Seine Bay and estuary and Gironde estuary (other studies Martin and coworkers).

α-emitting radionuclides, where the total α-radiation is about two to three orders of magnitude higher than the plutonium-α-radiation.

The 1980 results are presented in Table I, divided into three sections: Fresh salt-marsh sediment at Waarde and Borssele (Western Scheldt), old salt-marsh samples from Delta Area and seston (suspended matter) obtained by centrifugation of sea water (50 gram requires about 2 days shiptime).

The results are not yet related with the plutonium data since these are not yet complete.

Sub-Project 2. Association Euratom-ITAL
Head research team: Dr. F.I. Frissel
General subject : Analysis of Pu in field samples

The analyses for plutonium have been concentrated in 1980 on a few vegetation samples *(Aster tripolium)* and on a number of salt-marsh sediment samples from the stock of the Delta Institute at Yerseke. The results obtained for ^{239}Pu in *Aster tripolium* (Table II) agree with those obtained in 1979,but the ratio $^{238}Pu/^{239}Pu$ is higher (0.14-0.27) than the values found in salt-marsh sediments (0.08-0.14). At present these results need to be re-evaluated against other results, but the ^{239}Pu (normalized to clay) values for the sediments sampled from 1961 show that at one place (e.g. Springersgors) the concentrations have a maximum at or before 1968, related probably with fallout peaks from the bomb-testing series which ended in 1962.

Sub-Project 3. Laboratoire de Géologie, Paris
Head research team: Dr. J.M. Martin
General subject : Homogeneity tests of intercalibration samples, intercalibration programme and analyses Pu in field samples

As already mentioned in the introduction not all plutonium- and other radionuclide analyses have been completed for the 1980 samples. On the other hand a comparison can be made of the so-far obtained results with measurements from other areas.

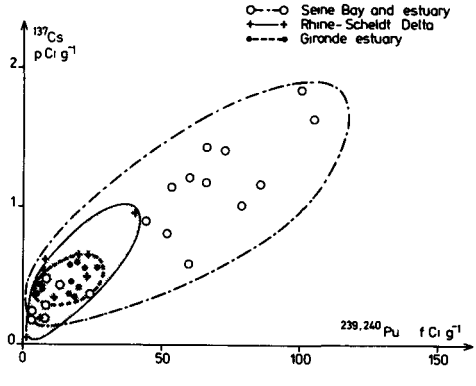
Table II. Results on Pu measurements in *Aster tripolium* and salt-marsh sediments sampled from 1961-1978 (in pCi kg^{-1})

Samples		$^{238}Pu/^{239}Pu$	^{239}Pu	^{238}Pu	Tracer recovery %	^{239}Pu normalized to clay
Aster, Ellewoutsdijk	(9'79)* (0)**	0.14	0.29	0.04	46	
Aster, Springersgors	(9'79) (7)	0.14	0.14	0.02	46	
Aster, Stroodorpepolder	(9'79) (6)	0.18	0.11	0.02	60	
Aster, Waarde	(9'79) (1)	0.27	0.41	0.11	84	
Kaloot	(5'62) (3)	0.08	2.5	0.2	32	13.2
Spieringschor	(7'62) (4)	0.05	17.9	0.9	32	31.8
Noordsloe	(8'62) (5)	0.14	0.7	0.1	13	1.9
Stroodorpepolder	(3'65) (6)	0.08	2.3	0.2	18	21.5
idem	(7'68) (6)	0.10	1.9	0.2	32	15.3
Springersgors	(8'62) (7)		18.0	?	41	59.8
idem	(7'68) (7)	0.06	53.7	3.3	47	165.2
idem	(7'70) (7)	0.05	36.4	1.9	22	61.8
idem	(10'72) (7)	0.06	34.3	2.3	33	57.6
Zuidland	(8'61) (8)	0.04	5.1	0.2	20	34.0
Salt marsh Bergen op Zoom	(3'64) (9)	0.5	0.2	0.1	33	13.3
idem	(6'75) (9)	0.1	9.4	0.9	18	52.2
idem	(5'78) (9)	0.09	6.5	0.6	12	24.4

*) 9'79 = Sept. 1979
**) (0) see Fig. 1

Table III. Average results of $^{239, 240}Pu$ and ^{137}Cs measurements in vegetation from different regions. The activities are normalized to the potassium contents of the plants in fCi g^{-1} potassium for $^{239, 240}Pu$ and pCi g^{-1} potassium for ^{137}Cs.

	$^{239,240}Pu$	^{137}Cs
Aster tripolium (Rhine-Scheldt delta)	12.5	54
Grass (Nord Cotentin Brittany)	34	4
Grass (La Hague area)	222	17



Vegetation

Specific activities, normalized to potassium of *Aster tripolium*, a salt-marsh plant also used as vegetable, have been compared to those of grass samples from 2 French areas located in North Cotentin and La Hague. The last location is near to the La Hague Nuclear Reprocessing Plant, while the first one is from an area only contaminated by atmospheric fall-out from the 1958-1962 nuclear bomb-testing.The results are summarized in Table III.

In all samples, the activities appear to be very low, especially those measured in *Aster tripolium* of the Rhine-Scheldt delta. These are very close to grass samples from the Northern Cotentin Brittany area, both primarly contaminated by fall-out from bomb-testing. It is worthwhile to note that the La Hague grass samples Pu activities are higher by more than one order of magnitude. This is mainly due to the atmospheric recycling of marine aerosols (MARTIN, THOMAS and JEANDEL, in press), rather than due to direct contamination.

A first assessement of the transfer coefficient between soils and *Aster tripolium* shows that less than 5% can be accumulated by this species.

Sediment samples

In Fig. 2 ^{137}Cs and $^{239-240}Pu$ activities of deposited and suspended sediments from the Rhine-Scheldt delta are compared to those of other estuarine environments (i) the Gironde estuary (which can be assumed as a background for atmospheric fall-out and (ii) the Seine Bay estuary (which is notably contaminated by the La Hague Centre and possibly by Windscale nuclear plant wastes).

For both isotopes, the Rhine-Meuse-Scheldt estuary samples show in general similar activities as compared to those measured in the Gironde estuary. A very few number of samples appear to have slightly higher concentrations. However, it must be kept in mind that due to the well known affinity of trace metals and radionuclides for clay-size fractions the specific activities representative of suspended sediments (i.e. with a similar matrix to the Seine and Gironde samples) might be higher and therefore probably intermediate between the fall-out level (Gironde) and the contaminated Seine estuary.

It is also expected that the general pattern of the plutonium geochemical behaviour in the Rhine-Scheldt delta might be similar to that observed in French estuaries, i.e. a systematic increase of particulate specific activity along with salinity (JEANDEL, MARTIN and THOMAS, 1980).

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Contractant de la Commission : Commissariat à l'Energie Atomique
Département de Protection/S.E.R.E. (France)

N° du contrat : 235-76-7 BIO F
Chef du (des) groupe (s) de recherche : Pierre BOVARD

Thème général du contrat : Projet commun de recherches sur le cycle de l'eau et celui du tritium

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Titre du projet n° 1 : ETUDE DU CYCLE DE L'EAU ET DU TRITIUM DANS LES ESPECES MEDITERRANEENNES

Chef du projet et collaborateurs scientifiques : Yves BELOT, Claude CAPUT,

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Les doses à l'homme résultant de rejets d'eau tritiée dans les systèmes aquatiques ou l'atmosphère ne peuvent être prédites correctement que si on connaît le comportement du tritium dans les différents maillons de la chaîne alimentaire. Il est nécessaire en particulier d'avoir des données sur le comportement du tritium dans le système sol-plante-atmosphère. Les travaux exécutés dans le cadre du présent contrat ont été principalement consacrés à des études sur le comportement du tritium dans une espèce méditerranéenne (*Vitis vinifera*) et à titre comparatif dans deux espèces plus largement répandues (*Zea mays et Solanum tuberosum*). Ces plantes ont été choisies en tant que composantes importantes de l'alimentation animale et humaine.

Transfert de l'eau tritiée entre l'atmosphère et les feuilles des plantes

Des expériences de terrain ont été réalisées pour étudier le transfert de la vapeur d'eau tritiée entre l'atmosphère et des plants de vigne cultivés in situ. La cinétique du transfert a été explorée en relation avec la résistance stomatique et la température des feuilles. Pour cela, des plants de vigne ont été exposés à des concentrations connues

de vapeur d'eau tritiée produites au moyen d'un dispositif mobile spécialement conçu pour cette expérience. Pendant et après l'exposition, des échantillons foliaires ont été prélevés à différents moments, et leur contenu en eau tritiée a été déterminé. En même temps, des mesures ont été faites de la résistance stomatique, de la température et de l'humidité relative de l'air ambiant.

Les résultats obtenus montrent que le transfert de l'eau tritiée entre l'atmosphère et les feuilles se fait par échange gazeux à travers les orifices stomatiques des feuilles. La captation, mais aussi la perte d'eau tritiée par la plante, sont plus rapides pendant le jour que pendant la nuit, et plus rapides au printemps qu'en automne. De façon générale les échanges d'eau tritiée sont d'autant plus actifs que les stomates sont plus ouverts, et que le flux de transpiration est plus important.

Dans le cas d'une plante exposée à une concentration constante de vapeur d'eau tritiée, on observe que l'activité de l'eau foliaire supposée nulle au départ, augmente avec le temps et se rapproche exponentiellement d'un état d'équilibre. L'activité de l'eau foliaire, en conditions d'équilibre, est en général beaucoup plus petite que l'activité de l'eau atmosphérique. Ce résultat indique qu'une partie de l'eau foliaire provient d'un échange avec la vapeur d'eau atmosphérique et qu'une autre partie provient de l'eau non contaminée du sol qui est apportée aux feuilles par le flux de transpiration.

Un modèle a pu être développé, qui tient compte des résultats expérimentaux ainsi acquis, et qui donne la concentration de tritium dans l'eau des feuilles en fonction du temps, connaissant la résistance stomatique et la température. Ce modèle permet une évaluation de la concentration atteinte à l'équilibre, et une évaluation de la période biologique du tritium contenu dans l'eau tissulaire. Ce modèle pourra être intégré dans un modèle plus vaste de dispersion du tritium au sein des divers compartiments du milieu naturel.

Incorporation du tritium dans la matière organique de plantes terrestres (en collaboration avec l'équipe du Dr Kirchmann).

Les deux dernières années du contrat ont été plus spécialement consacrées à une étude de l'incorporation du tritium dans la matière organique de plantes terrestres exposées à des rejets de vapeur d'eau tritiée de courte durée. Pour cela, des plants de vigne, maïs et pomme de terre ont été exposés pendant plusieurs heures à un courant d'air contenant de la vapeur d'eau tritiée, ainsi que du gaz carbonique marqué par le carbone-14. Le gaz carbonique radioactif a été utilisé comme marqueur de la photosynthèse. A la fin de la période d'exposition, les feuilles ont été collectées, le tritium et le carbone-14 ont été mesurés dans l'eau et dans la matière sèche des feuilles.

Ces essais ont montré que les quantités de tritium et de carbone-14 incorporées dans la matière organique des feuilles sont étroitement corrélées entre elles. Ce résultat démontre que l'incorporation dépend exclusivement de la photosynthèse et que la quantité incorporée est limitée par l'activité photosynthétique. C'est ainsi que dans des conditions de beau temps et par conséquent d'activité photosynthétique élevée, l'activité spécifique de la matière organique, après une heure d'exposition, est égale à 0,015 fois environ l'activité spécifique de l'eau. L'activité spécifique de la matière organique peut être presque nulle lorsque l'exposition se fait dans des conditions où la photosynthèse est très peu active ou même nulle. Ces résultats permettent une prédiction plus réaliste de l'incorporation du tritium en cas d'exposition accidentelle de courte durée. Les données obtenues seront publiées dans le courant de l'année 1981.

Publications

Bovard P., Belot Y., Delmas J., Camus H., Grauby A. Kirchmann R. and Van den Hoek J. (1979) "Transfer to the diet of tritium emitted by nuclear installations" Proceedings of a Symposium on the Behaviour of Tritium in the Environment, San Francisco, 1978. Paper IAEA-SM-232/99 p. 419, Vienna 1979.

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Contractant : Centre d'Etude de l'Energie Nucléaire

N° du Contrat : 236-77-1 BIO B
Chef de projet : Ir. R. KIRCHMANN

Titre du projet : Etude du cycle de l'eau et du tritium dans les régions tempérées.

Le présent rapport concerne les expériences et les résultats obtenus dans le cadre d'un programme de recherche mené au CEN/SCK en collaboration étroite et suivie avec le Service d'Etudes et Recherches sur l'Environnement Fontenay-aux-Roses, France et la Landbouw Hogeschool (Wageningen, Pays-Bas).

Les recherches conduites au CEN/SCK poursuivaient les objectifs initiaux suivants:

a) Etude de la contamination des végétaux comestibles, après dépôt d'eau tritiée (eau d'irrigation, vapeur d'eau atmosphérique);

b) Etude de l'incorporation du tritium dans les différents organes ou tissus d'animaux de ferme ayant ingéré du tritium, l'incorporation dans certains constituants cellulaires nécessitant la mise au point de techniques d'isolement et de purification (DNA).

1. Contamination des Végétaux

1.1. Végétaux consommés par l'homme

1.1.1. Dépôt unique d'eau tritiée

Ce type de dépot, représentant le cas de rejet accidentel ou concerté, a été appliqué au moment où le besoin en eau des végétaux étudiés (betterave, pomme de terre, pois, carotte) était le plus élevé. Des échantillons de sol et de végétaux ont été récoltés à intervalles déterminés depuis le moment du dépôt de l'eau tritiée jusque la récolte finale afin de déterminer l'incorporation du tritium dans les fractions de l'eau tissulaire et de la matière organique ainsi que la distribution de l'eau tritiée dans le profil du sol. Les valeurs du rapport de l'activité spécifique (pCi $^{3}H/gH$) dans la matière organique et dans l'eau tissulaire varient de 5,2 dans le grain de pois à 0,84 dans le tubercule de pomme de terre; ces résultats montrent que chez les espèces végétales étudiées, l'incorporation du tritium dans la fraction organique a un temps de résidence généralement plus long que celui du tritium contenu dans la fraction d'eau tissulaire

Par ailleurs dans la couche superficielle (0-10 cm) du sol, on observe un pic dans la teneur en ^{3}H de l'eau du sol quelques heures après le dépôt mais, un mois après le dépôt, la concentration dans cette couche de sol avait diminué d'un facteur 100 environ: par contre une infiltration dans les horizons plus profonds. Pour l'ensemble des parcelles cultivées, 41% du dépôt initial était encore présent dans le sol 39 jours plus tard dont 0,3% seulement dans la couche superficielle (0-10 cm). D'autre part une bonne corrélation a été observée entre la teneur de l'eau du sol superficiel

(0-10 cm) et la teneur en tritium de l'eau tissulaire de la partie aérienne des végétaux étudiés. Par contre aucune relation significative n'a été trouvée entre les teneurs en ^{3}H, de l'eau des tubercules de pommes de terre, des racines de betteraves et des grains de pois, et les teneurs en tritium de l'eau du sol jusqu'à une profondeur de 80 cm.

1.1.2. Fixation de tritium à partir de la vapeur d'eau atmosphérique (exposition unique)

En collaboration avec l'équipe de Fontenay-aux-Roses (Projet N° 1) qui a mis en oeuvre un fumigateur équipé d'un générateur de ^{3}H et de CO_2 (marqué au ^{14}C), des expériences sur des végétaux (pomme de terre et maïs) qui diffèrent par leur mécanisme de photosynthèse ont été réalisées en vue de rechercher la vitesse d'incorporation du tritium dans la matière sèche en fonction de l'activité photosynthétique (dans la partie aérienne). Après exposition (de 4 1/2h à 7h) les niveaux de tritium mesuré, chez les espèces végétales, dans l'eau tissulaire (TFWT) et dans la matière organique (OBT) étaient similaires.

1.1.3. Translocation et évolution du ^{3}H et ^{14}C fixés par les végétaux

Des expériences ont débuté en 1980 sur des végétaux (pommes de terre, maïs) cultivés sous abri plastique et exposés, au cours de la croissance, pendant quelques heures à une contamination unique par du gaz carbonique (^{14}C) et de la vapeur d'eau tritiée, de façon à contaminer le feuillage à un niveau relativement élevé permettant de suivre le transfert des radionucléides dans les autres compartiments du milieu. Les mesures de ^{3}H et de ^{14}C sur les échantillons de sol et des divers organes des végétaux sont en cours.

1.1.4. Transfert du ^{3}H des déchets organiques aux plantes cultivées

La forme initiale du tritium dans le sol (eau tritiée ou matière organique) ne semble pas influencer la répartition centésimale du tritium dans les organes des plantes récoltées (pommes de terre) ni le taux de transfert dans les tubercules lequel est d'environ 13%.

1.2. Fourrage tritié

Une parcelle (1440 m^2) de prairie à foin et une parcelle (864 m^2) de maïs fourrager ont été irriguées par de l'eau tritiée lorsque l'humidité du sol tombait en dessous des 2/3 de la capacité au champs. L'activité totale déposée a été de 4,5 Ci d'eau tritiée durant cette période; la production de foin a été de 277,8 kg (1250 pCi ^{3}H/g mat. sèche) et celle du maïs fourrager de 1 tonne (1350 pCi ^{3}H/g mat. sèche). Ces fourrages ont servi à nourrir des vaches laitières (voir projet 3) en vue d'étudier le transfert du tritium dans les constituants du lait.

2. Contamination des animaux de ferme

Le but des expériences réalisées est de rechercher le "devenir" du tritium ingeré, avec des aliments liquides ou solides, par des animaux de ferme.

2.1. Incorporation du tritium dans les macromolécules biologiques

Afin d'étudier la répartition du tritium dans les différents composants subcellulaires ainsi que dans le DNA lui-même et les autres composants étroitement liés à la macromolécule, il a été procédé à la mise au point de méthodes de préparation et d'analyse du DNA.

Deux méthodes de purification, respectivement ultracentrifugation en gradient CsCl et filtration moléculaire sur gel d'agarose, ont permis de préparer du DNA pouvant être considéré comme relativement pur et propre, contenant très peu de protéines et de traces de RNA.

2.2. Incorporation dans les organes du porc

2.2.1. Ingestion de pommes de terre tritiées, lyophilisées

Au total 12kg450 de pommes de terre tritiées et lyophilisées ont été administrés à un porcelet pendant 21 jours, ce qui représente une activité totale administrée de 42,5 µCi. L'excrétion fécale et urinaire a été suivie sur base d'un échantillonnage journalier de faeces et d'urine. Au sacrifice l'activité en tritium de l'eau tissulaire et de la matière organique de différents organes a été déterminée.

2.2.2. Ingestion d'eau tritiée chez la truie en gestation

Pregnant sows were given tritiated water during the entire pregnancy and for 43 days thereafter. The young pigs were, in part, left with their mother, in part, they were swapped with uncontaminated new-borns in order to follow tritium oxide and organic tritium in different organs with respect to continuing uptake after birth, uptake from milk, loss of activity after birth.

In summary, tritium oxide in pigs exposed continuously displays also a slow metabolic component representing not more than 5% of the total. About 70% of the body water is derived from ingested water, the rest originates from water in food or formed by metabolism. This value is about the same in adult and neonatal pigs. No difference is found between organs with respect to metabolism of tritium oxide between organs.

SUMMARY OF EXPERIMENTAL DESIGN

SOW	DOSE	WEIGHT	WATER CONSUMPTION AFTER 77 100 120 days	YOUNG	TREATMENT YOUNG PIGS
A	0,517 mCi/l	158 kg	535 l 752 l 975 l	A1	Kept with A, killed at birth
				A2	A, at 23 days age
				A3	A, at 43 days age
				A4	A, at 86 days age
				A5	Transferred to C, killed at 23 days age
				A6	C, at 43 days age
				A7	C, at 86 days age
B	1,532 mCi/l	139 kg	485 l 747 l 1022 l	B1	Kept with B, killed at birth
				B2	B, at 23 days age
				B3	B, at 43 days age
				B4	B, at 86 days age
				B5	Transferred to C, killed at 23 days age
				B6	C, at 43 days age
C	control	131 kg	(not determined)	C1	Kept with C, killed at birth
				C2	C, at 23 days age
				C3	Transferred to A, killed at 23 days age
				C4	A, at 43 days age
				C5	A, at 86 days age
				C6	Transferred to B, killed at 23 days age
				C7	B, at 43 days age
				C8	B, at 86 days age

Organically bound tritium attains an equilibrium value of about 11% of that of the tritium oxide given. Only brain has a significantly greater specific activity (about 17%) in agreement with observations by others authors. Turnover of organic tritium varies among organs, it is slowest in brain, followed by muscular tissues, it is most rapid in liver and intestine. Serum proteins have about the same turnover and the same equilibrium value as organs. From these values, one can estimate that continuous exposure to tritium oxide during pregnancy results in a dose from organic tritium which in most organs is only about one third to two third of that due to tritium oxide alone. In brain, this dose from organic tritium may, however, be as large ore even slightly exceed that from tritium oxide.

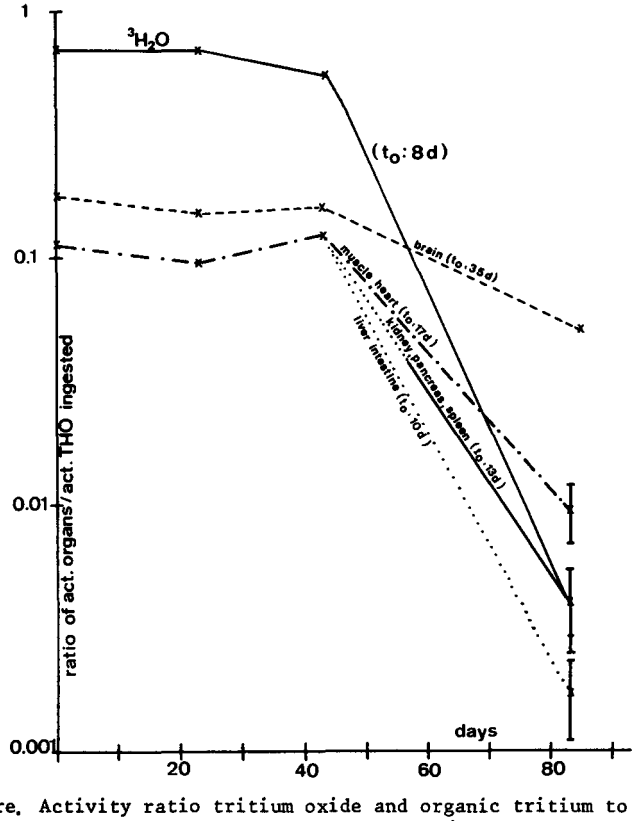

Figure. Activity ratio tritium oxide and organic tritium to tritium oxide ingested in organs of young pigs whose mother had received tritium oxide and which during lactation were maintained with the contaminated mother but later given noncontaminated water.

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BOVARD P., BELOT Y., DELMAS J., CAMUS H., GRAUBY A., KIRCHMANN R., VAN DEN HOEK J. - Transfer to the diet of tritium emitted by nuclear installations. Proceedings of a Symposium on the Behaviour of Tritium in the Environment, San Francisco, 1978. Paper IAEA-SM-232/99, p. 419, Vienna 1979.

VAN DEN HOEK J., KIRCHMANN R., VAN BRUWAENE R. - Tritium contamination of milk after ingestion of tritiated forage (corn). Proceedings of the 10th E.S.N.A. Annual Meeting, Belgrade 1979, Environmental Pollution Working Group News Letter, p. 9.

VAN DEN HOEK J., GERBER G.B., KIRCHMANN R. - Tritium in organic constituents of cow's milk and in milk water after ingestion of organically bound tritium. Proceedings of a EULEP Symposium on "The Metabolism and Toxicity of Tritium", London, 1980, EULEP Newsletter.

VAN DEN HOEK J., GERBER G.B., KIRCHMANN R. - Excretion of organic and inorganic tritiated compounds in cow's milk after ingestion of tritium oxide. Proceedings of the 5th IRPA Congress, Jerusalem 1980, Volume III, p. 289.

Contractant of the Commission: Landbouwhogeschool, Wageningen, Netherlands

No. of the contract: 237-76-7 Bio N
Head of the research team: Dr. Jan VAN DEN HOEK

Title of the project: METABOLISM AND BEHAVIOUR OF THO AND OF H_2O IN HERBIVOROUS ANIMALS

The metabolism of tritium has been studied in lactating cows after administration of tritiated water and of organically bound tritium (tritiated corn and tritiated hay). Milk was chosen as the product in which tritium metabolism was to be investigated. There are two main reasons for this choice. Firstly, milk is an important animal metabolite in which a protein (casein), a fat (milk fat) and a carbohydrate (lactose) are newly synthesized continuously. Incorporation of tritium in these important macromolecules can thus be studied in relation to the chemical form in which tritium is administered to the animal. Secondly, milk is an important food item and has a particular significance as a vector of radionuclides to man. The digestive processes in a polygastric herbivorous animal such as the cow, are so particular in a number of ways that the results obtained in this study can not always be extrapolated to monogastric animals, including man.

Tritium in milk after administration of THO

Tritium from orally ingested THO becomes incorporated into all the organic constituents of cow's milk which have been studied. At steady state conditions, milk water has the highest concentration of tritium which amounts to about 83% of the tritium concentration of the ingested water. As far as the organic compounds are concerned, the highest tritium concentration (pCi ^3H/g of product) is found in milk fat, closely followed by lactose whereas about half the amount of tritium is present in casein.

Our data, obtained in 2 animals, show only minor differences. It can be concluded from these data that, when tritiated water of constant specific activity is ingested by a lactating cow, from 1.5-1.7% of the daily ingested tritium is secreted in each litre of milk. Of this, no more than 5% is organically bound, the remaining 95% is in milk water. It follows that

the dose to man which results from drinking milk from cows which have been drinking tritiated water, comes predominantly from the THO in milk.

When the administration of THO is stopped, tritium activity in milk water and also in organic molecules decreases rapidly. The half-life for the fast component is 3.6 days for water and lactose, 4.0 days for casein and 4.1 days for milk fat. The long-lived components are 43.8 days for milk water, 224.8 days for milk fat and 18.2 days for casein. The contribution of the long-lived components to the total dose represent about 4% of the total dose.

Tritium in milk after ingestion of tritiated corn

When feeding non-dehydrated tritiated food to an animal, it ingests both THO and organically bound tritium (OBT). The resulting tritium levels in the tissues and products from the animal will depend on several variables such as the water content of the food, its content on precursors for synthesis of organic molecules and so on.

In the case of tritiated corn, milk fat was found to have the highest specific activity (pCi $^{3}H/g$ H), followed by milk water. The specific activities of casein and lactose were similar and about 60% of the value found for milk fat. Since the water content of milk is so high (about 87%), the dose to man, also in this case, will be derived primarily from ingested THO although the contribution from organic compounds in milk will be more important than in the case of milk from cows ingesting tritiated water.

Tritium in milk after ingestion of tritiated hay

Tritiated hay had been obtained from grass which was repeatedly sprayed with tritiated water during its growing period. After complete evacuation of the THO, the hay was administered twice daily to a lactating cow until near steady state conditions had been obtained.

The highest tritium activity was found in milk fat. It was about 2.5 times the activity in casein and about 16 times that in lactose. The tritium activity in milk water was low and comparable to that in lactose.

The transfer of the daily ingested tritium, secreted per litre of milk, can be estimated to range between 1.0-1.5%. More than half the tritium is found in milk water on a per litre basis and about one third in milk fat.

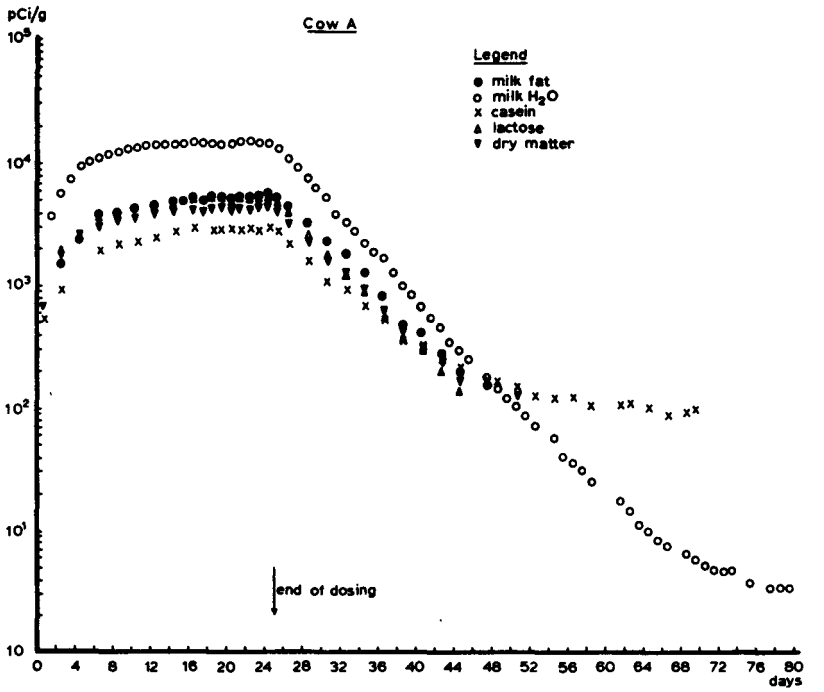

Figure 1. The evolution of Tritium activity in milk water and in some organic milk constituents after continuous ingestion of tritiated water by a lactating cow for 25 days.

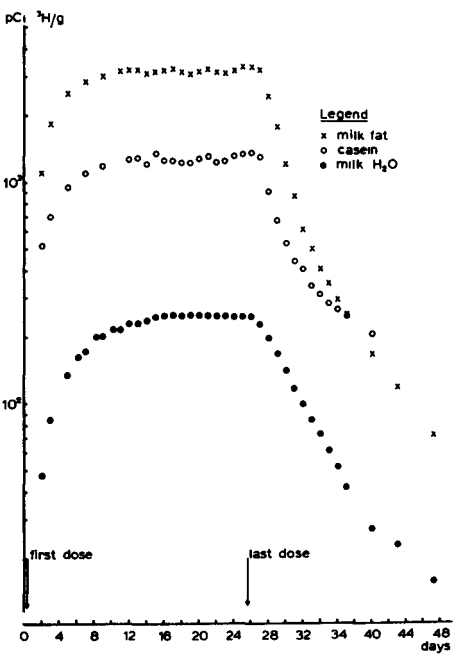

Figure 2. The evolution of Tritium activity in some organic milk constituents and in milk water after continuous administration of organically bound tritium (tritiated hay) to a lactating cow for 25 days.

When the administration of tritiated hay is stopped, tritium in organic compounds decreases very rapidly initially but much more slowly later. The disappearance curve of tritium in milk fat can be represented by three components with half lives of 2.9 days, 13.3 days and 89 days respectively. The long-lived component represents only 2% of the total tritium activity.

Summarizing the results, it can be concluded that the objectives, outlined at the beginning of the contract period, have been achieved. Sufficient data have accumulated to understand the water or grass-milk-man pathway for tritium.

VAN DEN HOEK, J., KORANDA, J.J., MORGAN, G.B., KIRCHMANN, R., BUSTAMANTE-JUAN, N. Transfer and incorporation of tritium in mammals. Proceedings of a Symposium on the Behaviour of Tritium in the Environment, San Francisco, 1978. Paper IAEA-SM-232/48, p. 433, Vienna 1979.

BOVARD, P., BELOT, Y., DELMAS, J., CAMUS, H., GRAUBY, A., KIRCHMANN, R., VAN DEN HOEK, J. Transfer to the diet of tritium emitted by nuclear installations. Proceedings of a Symposium on the Behaviour of Tritium in the Environment, San Francisco, 1978. Paper IAEA-SM-232/99, p. 419, Vienna 1979.

VAN DEN HOEK, J., KIRCHMANN, R., VAN BRUWAENE, R. Tritium contamination of milk after ingestion of tritiated forage (corn). Proceedings of the 10th E.S.N.A. Annual Meeting, Belgrade 1979, Environmental Pollution Working Group News Letter, p. 9.

VAN DEN HOEK, J., GERBER, G.B., KIRCHMANN, R. Tritium in organic constituents of cow's milk and in milk water after ingestion of organically bound tritium. Proceedings of a EULEP Symposium on "The Metabolism and Toxicity of Tritium", London, 1980, EULEP Newsletter.

VAN DEN HOEK, J., GERBER, G.B., KIRCHMANN, R. Excretion of organic and inorganic tritiated compounds in cow's milk after ingestion of tritium oxide. Proceedings of the 5th IRPA Congress, Jerusalem 1980, Volume III, p. 289.

Contractant de la Commission : Biologie, Radioprotection et Recherche Médicale XII 4 B

N° du Contrat : 231 - 76 - 7 - BIO F - VEN 1201 - 01
Chef du (des) groupe (s) de recherche : GRAUBY A. DPr/SERE
Thème général du contrat : Transfert du Pu dans l'environnement sous climat tempéré

Titre du projet n° 1

Chef du projet et collaborateurs scientifiques : SAAS A.
BOURREAU C. - GILLE M. - MORELLO M.

L'étude de l'évolution des transuraniens en particulier du plutonium dans l'environnement terrestre a été abordée sous plusieurs aspects : mise au point d'une méthode d'extraction et de dosage ; définition de la solubilité des transuraniens dans les eaux d'irrigation ; transfert sol-plante à long terme ; transfert en fonction du dépôt et évolution des transuraniens dans les sols.

1 - EXTRACTION ET DOSAGE DES ACTINIDES DANS LES SOLS ET LES VEGETAUX

L'expérimentation du transfert sol-plante sur les actinides a nécessité dans un premier temps la mise au point d'une méthode d'extraction et de dosage pour des quantités allant de 1 pCi à 100 pCi par échantillon. Les méthodes utilisées pour l'environnement sont certes très sensibles mais de mise en oeuvre longue et souvent spécifique à un isotope ou groupe d'isotope. La technique retenue consiste d'abord à extraire les isotopes du milieu par voie humide (HNO_3 - HF), de fixer ensuite les transuraniens sur une colonne de bille de verre ; l'élution à l'aide d'une solution acide est suivie d'une précipitation au fluorure de lanthane sur filtre millipore ; la détection se fait à l'aide d'une chambre à grille. Les figures suivantes illustrent quelques résultats obtenus sur des sols renfermant un mélange de 4 isotopes.

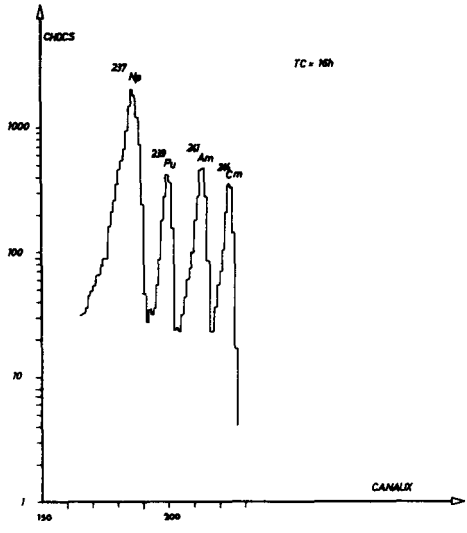

FIGURE 1 : Spectre obtenu par séparation sur billes de verre, des émetteurs alpha extraits par 50 ml d'acide acétique a 2,5 % sur 5 g de sol renfermant du neptunium 237, du plutonium 239, de l'américium 241, du curium 244.

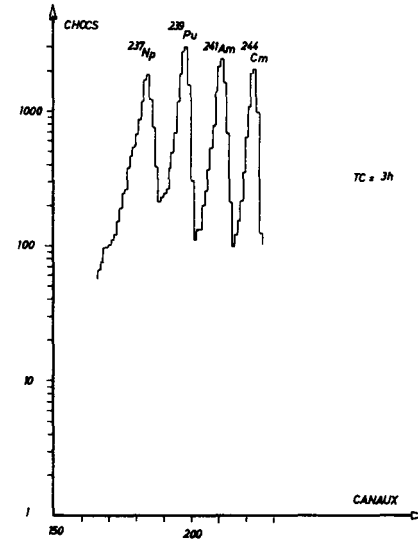

FIGURE 2 : Spectre obtenu par séparation sur billes de verrre, des émetteurs alpha extraits par 50 ml de pyrophosphate de sodium sur 5 g de sol renfermant du neptunium 237, du plutonium 239, de l'américium 241, du curium 244.

2 - DEFINITION DE LA SOLUBILITE DES TRANSURANIENS DANS LES EAUX D'IRRIGATION

Les expérimentations poursuivies sur des cultures submergées (riz) ont montré un passage relativement important au niveau des basses tiges (submergées) et aux racines. Ceci ne peut être lié qu'à la présence de formes complexées ou pseudosolubles. Nous avons donc suivie le potentiel de mobilité des transuraniens dans les eaux superficielles. Une fraction allant de 1 à 10 % est plus ou moins soluble dans les eaux et peut migrer même sur gel de silice en milieu neutre. Cette fraction migre dans le sol et est transférée au sol par les eaux d'irrigation. La présence de composés organiques favorise la complexation et la mobilité des transuraniens en milieu terrestre.

3 - TRANSFERT SOL-PLANTE A LONG TERME POUR LES TRANSURANIENS

Les essais de transfert à long terme pour les actinides se sont poursuivis sur 4 ans afin de définir avec précision les valeurs à prendre en considération sur le plan de la radioprotection. Dans nos conditions expérimentales (sol brun calcaire - haricot

vert et haricot sec) les taux de transfert obtenus sont rassemblés dans le tableau ci-après.

TABLEAU I: Facteurs de transfert des actinides après 4 ans de culture

Isotope	Transfert aux parties aériennes	Transfert aux fruits
Plutonium	5.10^{-3} à 10^{-3}	10^{-4} à 10^{-3}
Américium	5.10^{-2} à 10^{-2}	10^{-3} à 10^{-2}
Curium	5.10^{-2} à 10^{-2}	10^{-3} à 10^{-2}
Neptunium	5.10^{-2} à 10^{-1}	5.10^{-3} à 10^{-2}

L'évolution du transfert du plutonium en fonction du nombre de culture est illustré par la figure 3.

Il apparaît par ailleurs qu'il existe un effet de synergie entre les différents transuraniens dûs d'une part à l'activité spécifique des isotopes et d'autre part au potentiel d'oxydo-réduction. De plus, la matrice uranium-thorium qui accompagne les rejets est capable de modifier partiellement le transfert sol-plante.

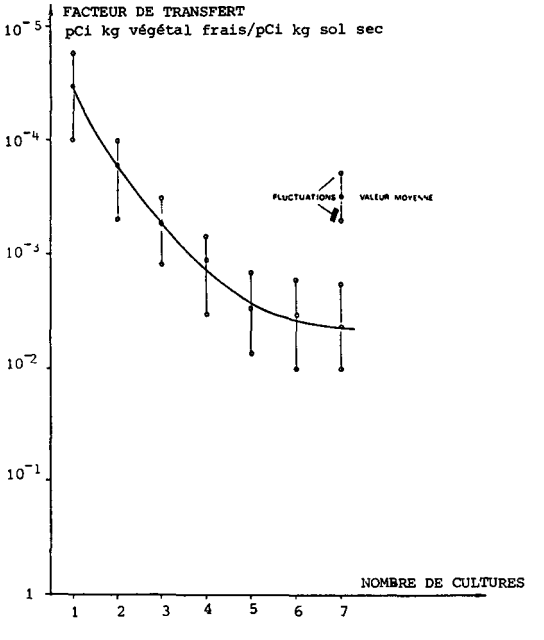

FIGURE 3 : Evolution du facteur de transfert du plutonium dans le haricot frais en fonction du nombre de cultures.

4 - TRANSFERT SOL-PLANTE DES TRANSURANIENS EN FONCTION DU DEPOT AU SOL

Le transfert sol-plante mesuré dans diverses conditions notamment à l'aide d'apport au sol élevé (µCi/kg de sol) faisait apparaître des valeurs plus faibles (10^{-5}) que celles obtenues avec des dépôts peu importants (pCi-nCi/kg de sol). Nous avons donc testé pour un même sol, le passage au haricot vert du plutonium pour différents apports. Les valeurs rassemblées dans le tableau II, permettent de constater que le dépôt joue effectivement un rôle non négligeable ; cet effet peut être attribué à l'évolution physico-chimique dans le sol et une migration rapide des formes complexées et pseudo-solubles.

TABLEAU II: Facteur de transfert en fonction du dépôt

Dépôt au sol (nCi/kg de sol)	Facteur de transfert $\frac{\text{pCi/kg haricot vert}}{\text{PCi/kg sol sec}}$
0,1	10^{-3}
1	8.10^{-4}
10	3.10^{-4}
50	8.10^{-5}
100	7.10^{-5}

5 - EVOLUTION DU DEPOT DE TRANSURANIENS DANS LES SOLS

Après 4 années de culture, la détermination des formes physico-chimiques par extraction successive a permis de mettre en évidence qu'une certaine quantité restait biodisponible. Sur sol calcaire les valeurs suivantes ont été obtenues.

TABLEAU III: Biodisponibilité et fraction organique

Isotope	% biodisponible (Ac.Acét.2.5%)	% organique (NaoH-PyroNa)
Plutonium	0,8	28,0
Américium	1,3	31,0
Curium	0,8	22,0
Neptunium	4,4	20,0

On constate que le neptunium est plus disponible que les autres transuraniens ce qui est confirmé par les valeurs de transfert plus élevés pour cet isotope. Par ailleurs, sauf pour le neptunium, la fraction incorporée dans la matière organique extractible (acides humiques et fulviques) est 20 à 30 fois supérieure à la fraction assimilable. La minéralisation des composés organiques par voie microbienne peut donc fournir à long terme des isotopes directement assimilables par les végétaux.

6 - CONCLUSIONS

Sur le plan de la radioprotection, l'ensemble de cette étude expérimentale apporte une contribution importante sur les points suivants :

- présence de formes solubles ou pseudosolubles dans les eaux superficielles,
- évolution dans le temps du transfert sol-plante,
- accroissement de la biodisponibilité dans le sol lié au rôle de la matière organique et de la vie microbienne,
- influence du dépôt sur le transfert sol-plante.

7 - LISTE DES PUBLICATIONS EN COURS

1) Extraction et dosage des émetteurs alpha en mélange dans des conditions expérimentales.

2) Potentiel de mobilité des transuraniens dans les eaux de fleuve. Health Physics sous presse.

3) Transfert à long terme des transuraniens dans les végétaux.

4) Transfert sol-plante des transuraniens en fonction du dépôt au sol.

5) Formes physico-chimiques des transuraniens dans un sol brun calcaire.

Vertragspartner der Kommission: Gesellschaft für Strahlen- und Umweltforschung München

Nr. des Vertrags: 260-77-7 BIO D
Leiter der Forschungsgruppe(n): Prof.Dr.W.Kühn

Allgemeines Thema des Vertrags: Inventory of Iodine-129 in the Thyroid Glands of Pasture Animals in the European Communities.

Titel des Projekts Nr. 1.

Inventory of Iodine-129 in the Thyroid Glands of Pasture Animals in the European Communities.

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof.Dr.W.Kühn, Dr. J. Handl

The primordial unstable iodine-129 with a half-life of 1.7×10^7 years has decayed. However, in the earth crust a considerable number of fission products including I-129 are formed by spontaneous fission of the actinides incl.U-238.It is found in the earth crust at a mean activity concentration of 75 f Ci/kg. Additionally , I-129 is produced in spallation processes of xenon in the high atmosphere by the interaction of primary cosmic radiation with xenon. It is also generated by neutron capture reactions from tellurium. On the basis of estimations of all production processes an equilibrium ratio of I-129/I-127 of about 3×10^{-14} is assumed in the hydrosphere, lithosphere and biosphere.

Human thyroid glands from 1936 showed an isotope ratio of I-129/I-127 $< 4 \times 10^{-11}$.Since the beginning of the nuclear age, approximately since 1945, larger amounts of iodine-129 are produced by neutron-induced fission of U-235 and Pu-239. About 12Ci have been generated by tests of nuclear weapons. The production rate of I-129 in reactors (regardless the reactor type) by fission processes is about 0.4Ci/GW(e) x year, and it is released into the atmosphere mainly during the reprocessing of spent fuel. As a result of this the isotope ratio of I-129/I-127 increased in the USA, for example, between 1950 and 1952 to a value of $\approx 10^{-6}$, measured near reprocessing plants. The ratio also increased in the thyroid glands of pasture animals to 10^{-8} and 10^{-7} even at greater distances from the emitting source. In comparison with the pre-nuclear times the present isotope ratio found in pasture animals (cattle) and in man in the USA is given as follows:

Pre-nuclear times :	$1.5 \times 10^{-14} <$ I-129/I-127 $\leq 9.8 \times 10^{-14}$
Biosphere (1947), USA:	I - 129 / I - 127 = 7.6×10^{-10}
Missouri (Biosphere) :	I - 129 / I - 127 = 5.9×10^{-9} in cattle
Missouri (Biosphere) :	I - 129 / I - 127 = 2.3×10^{-9} in man

The investigations on the appearance of iodine-129 in the vicinity of nuclear installation and especially near reprocessing plants showed that it is necessary to determine the transfer of iodine-129 through the food chain. While the different paths and the burden resulting from iodine-131 have been studied extensively, essential informations on the large scale ecological behaviour of iodine-129 are still missing.

The aim of the work is concerned with the stocktaking of iodine-129 in the thyroids of pasture animals at selected sites of the European Communities, where reprocessing plants are planned or already in operation.

To coordinate the cooperation of the different institutions of the Communities a meeting took place in Hannover. The participants from different countries decided on a uniform procedure of collection and preparation of the thyroids around nuclear power and reprocessing plants and free areas in certain distances from the plants. For the purpose of the measurement a sufficently sensitive and relatively quick method had to be developed for I-129 and I-127, so that for each thyroid gland the results could be given as the ratio of both isotopes. The extraction procedure of iodine from the thyroid glands of pasture animals and the method to determine the iodine-129 has been selected under the point of view that after about 1947 the isotope ratios of I-129/I-127 generally reached a value of 10^{-9} to 10^{-8}. Therefore, the absolute limit of the sensitivity for the whole method was fixed at about these values. The method outlined in detail in (1) consists of a chemical extraction with adsorption of the iodine at a special kind of charcoal and activation analysis by thermal neutrons with gammaspectroscopy. During the running time of the project from July, 1977, to the end of 1980, the method was developed, and 77 thyroid glands from pasture animals have been measured. The results obtained in the different countries in the Communities are given in Table 1 referring to animals.

In addition to this 4 thyroid glands of bovines were cut into about 300 pieces each, and the distribution of the total iodine in the thyroid glands was measured by activation analysis. The same procedure was carried out with 4 human thyroid glands. This way one gets the quantitative distribution of the iodine-127 in the organ. Fig.1 shows how iodine is typically distributed in the bovine, while Fig.2 refers to human thyroid glands. For that purpose more than about 2000 activationanalytical determinations have been carried out.

TABLE 1

Iodine-129 in Thyroid Glands of Bovines (1978)

Origin	Place	Number of Samples	Distance to the nearest nuclear installation	Age (Month)	Sex	Isotope ratio $^{129}I / ^{127}I$
F R G	Mariensee (Lower Saxony)	36	> 250 km	18	-	$< 9.7 \cdot 10^{-9}$
Belgium	Retie	1	5,5 km (Mol)	--	-	$3 \cdot 10^{-8}$
		1	5,5 km (Mol)	--	-	$7,8 \cdot 10^{-7}$
		1	5,5 km (Mol)	--	-	$3,5 \cdot 10^{-7}$
	Balen	1	7,0 km (Mol)	--	-	$< 6,6 \cdot 10^{-9}$
	Pay de Herve	10	80 km (Mol)	--	-	$< 1,5 \cdot 10^{-8}$
	Gul ++)	1	7,0 km (Mol)	--	-	
The Netherlands x			6,5 km (Borssele)	24	m	
	Hewedorp	3	100 km (Mol)	24	m	$< 7,8 \cdot 10^{-9}$
			191 km (Jülich)	24	m	
			2,5 km (Borssele)	24	m	
	Ovezande	3	94 km (Mol)	24	m	$< 9,6 \cdot 10^{-9}$
			184 km (Jülich)	24	m	
			150 km (Borssele)	18	m	
	Meersen	5	58 km (Mol)	18	m	$< 1,5 \cdot 10^{-8}$
			41 km (Jülich)	18	m	
			142 km (Borssele)	18/21	m	
	Meyel	5	57 km (Mol)	18/21	m	$< 1,7 \cdot 10^{-8}$
			57 km (Jülich	18/21	m	
	Nederwert		45 km (Mol)	--	-	
		1				$< 1 \cdot 10^{-8}$
			60 km (Jülich)	--	-	
Italy						
	Roncarolo	10	--	--	-	$< 5,4 \cdot 10^{-9}$
	Bobbio +)	4	--	--	-	

+) could not be treated till now
++) could not be treated because of technical reasons
x 15 further Thyroid Glands are under investigation at present

Bovine Thyroid

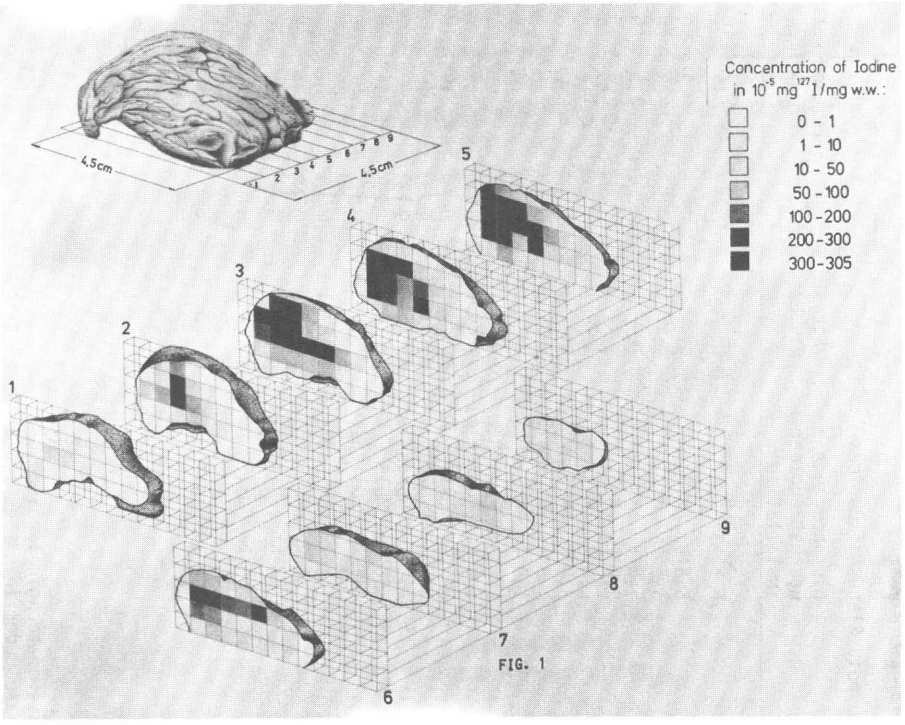

FIG. 1

Human Thyroid

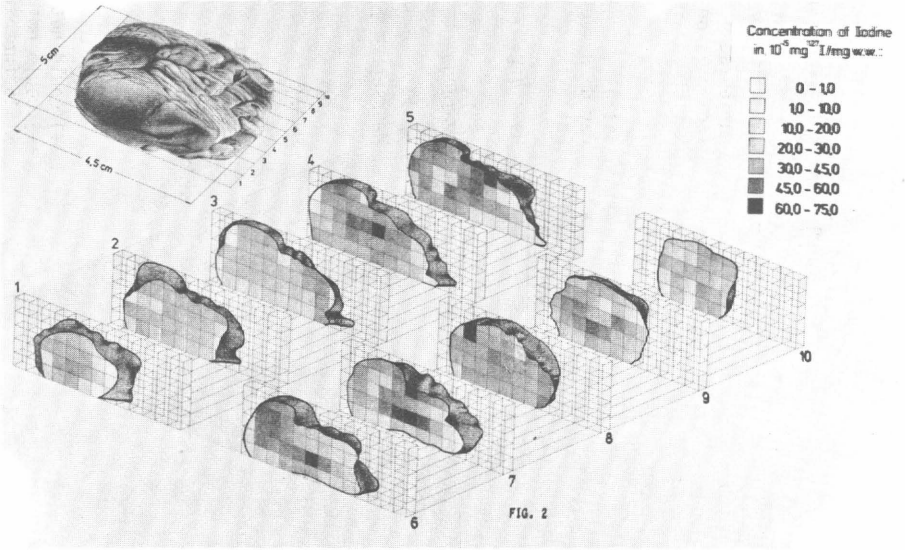

FIG. 2

Discussion:

The investigations on the stocktaking of iodine-129 in some countries of the European Communities show that the concentrations are generally in the same range as those measured in the USA with cattle and humans. This suggests the existence of a worldwide equilibrium level, which can be considered valid also for the Communities. There are, however, certain areas, e.g. around Mol in Belgium, where higher concentrations are found. This may be due to the spent fuel (≈200t), which has been reprocessed there until 1975. As only there and only up to a distance of 5.5 km from the plant I-129 was found, it can be concluded that in the near vicinity of such plants I-129 levels above the mean value have to be expected, and that I-129 is still present in the vicinity, at least several years after shut down of the plant. The investigations show the necessity of an I-129 stocktaking in human and animal thyroid glands expecially in areas, where reprocessing plants are planned or in operation.

The iodine distribution in thyroid glands exhibits an uneven incorporation of the element into the organ. This phenomenon has not been studied endocrinologically until now. Therefore, we suggest to continue these investigations to be able to draw medical or radiological conclusions from this observation.

The following institutions participated in the delivery of thyroid glands:

1. Centre D'Etude de L'Energie Nucleaire
   C.E.N./S.C.K. Mol, Belgique.
2. Institute for Atomic Sciences in Agriculture
   Wageningen, The Netherlands.
3. Comitato Nationale Per L'Energia Nucleare, Roma
   Centro Di Studie Nucleari, Della Casaccia, Italy.
4. Forschungsanstalt für Landwirtschaft, Mariensee, FR-Germany
5. Medizinische Hochschule, Hannover, Inst.f.Pathologie, FR-Germany

Publications:

(1) Handl,J., Kühn,W.: Activation Analytical Determination of Iodine-129 in Bovine-Thyroid Glands. J. of Radioanalytical Chemistry 56, 213-219,(1980)

(2) Handl,J., Kühn,W.: Jod-129 im Biozyklus; Statusbericht über den Transfer von Radionukliden 1980; Bundesministerium des Innern (1980).

(3) Glubrecht, H., Handl,J. and Kühn,W.: Studies on I-129 in Bovine Thyroid Glands and Investigations on Remobilisation of Iodine. Proc. of Iodine-129-Symp. Chiba, Japan, 1980

Contractor :	The Royal Veterinary and Agricultural University, Copenhagen.
Contract No. :	269-79-3 BIO DK
Head of the research team:	Niels Erik Nielsen
General subject of Contract :	Iodine uptake by plant roots with special reference to I-129 in the environment.

Title of project no. 1 : The kinetics of iodine uptake by plants in water culture.

Project leader and scientific staff : Henning Kaufholz

1.1. Introduction.

Small quantities of radioactive iodine, produced in nuclear reactors, may be released to the environment from nuclear fuel reprocessing plants. Radioactive iodine, especially I-129 (half-life = $1.7\ 10^7$ years), accumulated in the food-chain may ultimately result in radiation damage to the human thyroid. Plant and soil factors governing iodine uptake via the soil-soil solution-root-top pathway are rather incompletely known. Doubt has, therefore, been thrown on the validity of the accumulation factor (0.02 pCi I/kg fresh plant per pCi I/kg dry soil) normally used in the prediction of I-129 accumulation via the soil-soil solution-root-top pathway.

As the concentration of iodine, dissolved in the soil solution, is very low, i.e. less than 10^{-5} M, the rate determining step in the uptake by the plant is presumably located at the active root surface. In this case the rate of iodine uptake by plants from soil will depend on the factors: a) the area of the active root surface, b) the concentration of iodine in the soil solution at the active root surface, and c) the conductivity of the active root surface for iodine. (Nielsen 1972).

The project contained a study conducted in order to show if the factors a to c determine the rate of iodide and iodate uptake by intact plants from a nutrient solution of approximately the same composition as the soil solution of a fertile agricultural soil. In addition it was planned, but not realized, to investigate methyl iodide uptake by plants.

1.2. Materials and methods.

Barley (Hordeum vulgare L. var. Nordal) was grown in a growth chamber in water culture with a balanced nutrient solution excluding iodine. When the plants were five to seven weeks old batches of nineteen plants were transferred to 1.5 l nutrient solution with a low concentration of iodide or iodate kept aerated. The concentrations of iodide and iodate in the nutrient solution during depletion were determined. The concentrations of ions in the nutrient solution except for iodine were maintained constant during depletion by frequent ajustments. The volume of solution was maintained constant during depletion by addition of water from a Mariotte's bottle. Iodine was determined by

a catalytic spectrophotometric method or by an iodide electrode.

1.3. Results.

In order to obtain a curve fitting the experimental results and expressing concentration of iodide in solution as a function of time, the parameters of a logistic equation were optimized by use of a non-linear curve fitting procedure. From this equation and its first derivative values were computed respectively for the concentration of iodide in solution and for the rate of depletion of iodide from solution at various times during in the experiment. Rates of iodide uptake per unit of root length were calculated from the rates of depletion of iodide from solution. Corrections were introduced as well for iodide taken out for chemical analysis during the experiment as for increase in root length during the experiment. An exponential increase in root length was assumed.

In an analysis of the results iodide net uptake rate per unit of root length was related to the concentration of iodide in solution by the equation:

$$I_n = \frac{I_{max} \quad (c - c_{min})}{K_m + c - c_{min}} \qquad (1).$$

I_n denotes net influx or net uptake rate per unit of root length (f moles cm^{-1} sec^{-1}), I_{max} and K_m are maximal net influx (fmoles cm^{-1} sec^{-1}) and Michaelis-Menten constant for net influx of iodide (μM I^-) respectively. c is the concentration of iodide in solution (μM), and c_{min} is the concentration of iodide in solution at which $I_n \overset{\sim}{=} 0$ (μM). $K_m = c - c_{min}$ where $I_n = \frac{1}{2} I_{max}$. Values of I_{max} and K_m, obtained by a non-linear curve fitting procedure, and c_{min}, mean value of the concentration of iodide in solution during several hours of depletion after net uptake of iodide had ceased, are listed in table 1. Table 1 shows, that a lowering of the pH-value of the depletion solution from about 7 to about 4 effected an eighteen fold increase in the I_{max}-value and a 2.5-fold decrease in the c_{min}-value. The value of K_m did not change significantly with the pH-value of the solution. The coefficient of variance values in table 1 are associated with the variation between experiments.

Net influx parameters were not computed from the iodate depletion experiments. Instead, the values of iodate net influx at 0.4 μM iodate in solution were determined at pH 4.2 and pH 6.2. These net influx values are listed in table 2 together with the values of iodide net influx at 0.4 μM iodide in solution at pH 4.2 and pH 6.9. Net influx of iodate increased when the pH-value of the nutrient solution decreased. Compared at the same pH-value the net influx of iodate was only about 5% of the net influx of iodide.

1.4. Discussion and conclusion.

Results by Selders and Rediske (1954) showed that iodide uptake by plants in

nutrient solution culture was less from neutral than from acid solution (pH 4-7). Present results agree with this. Furthermore, if the kinetics of iodide uptake by intact plants is expressed in a Michaelis-Menten type relation it was found that only the parameters I_{max} and c_{min} are non-trivial functions of the pH-value of the nutrient solution. Both the fall in I_{max} and the increase in c_{min}, observed when the pH-value increases, will cause I_n to decrease according to equation 1.

Iodide concentrations in solutions were found below which net uptake of iodide by plants ceases. However, additional studies are needed in order to confirm that iodide accumulation by plants from soil during a growth period is nil when the concentration of iodide in the soil solution equals or is less than c_{min}.

Iodide net uptake parameters obtained in the present kinetics study are important plant factors in models used to simulate iodine and I-129 uptake by plants from soil. Agreement between predicted and observed uptake of I-129 by plants from soil can only be achieved if reliable values of plant and soil factors, governing iodine uptake by plants from soil, are known.

1.5. References.

Nielsen, Niels Erik 1972. Plant and Soil 36, 505-520.

Selders, A.A.; Rediske, J.H. 1954. U.S.-report HW-33681.

Table 1 Iodide net uptake characteristics of barley plants grown in solution culture for 35 or 49 days.

Exp. No.	pH-value	I_{max} *)	K_m	C_{min}
			µM	
1 (35 days)	4.2	2.4	1.1	0.06
2 (35 days)	6.9	0.15	0.9	ND **)
3 (49 days)	7.3	0.13	1.1	0.15
C.V. ***)		40	23	7.7

*) fmoles cm^{-1} sec^{-1}

**) ND = not determined

***) $CV = 100\, s_x / (\bar{x}\sqrt{2})$

Table 2. Net influx, I_n, of iodide (I^-) and iodate (IO_3^-) into barley roots at 0.4 µM iodide or iodate in solution.

Exp. No.	pH-value	Chemical form	I_n *)
1	4.2	I^-	57.0 10^{-2}
2	6.9	I^-	3.2 10^{-2}
4	4.2	IO_3^-	3.0 10^{-2}
5	6.2	IO_3^-	0.12 10^{-2}

*) fmoles cm^{-1} sec^{-1}

Table 3. Shoot and root characteristics of barley plants measured immediately after determination of iodine uptake characteristics in solution culture.

Exp. No.	Dry weight		Root length per g of		Average root radius r_o
	Shoot	Root	Root	Plant	
	g/plant		m/g		cm
1	2.47	0.25	616	57	0.0041
2	2.58	0.26	561	52	0.0041
3	3.33	0.28	467	37	0.0050
4	2.35	0.32	485	56	0.0043
5	2.86	0.32	471	53	0.0041
C.V. *)	5.6	9.9	5.0	6.1	2.3
n **)	34	1.8	1.8	1.8	1.8

*) CV = $100\, s_x / (\bar{x}\sqrt{n})$

**) n = average number of replicates.

Title of Project no. 2 : The kinetics of iodine uptake by plants from soil.

Project leader and scientific staff : Henning Kaufholz

2.1. Introduction.

Studies on the kinetics of iodine uptake by plants in nutrient solution culture may provide plant factors (parameter values) which can be applied in models to be used in predicting the quantity of I-129 accumulated by plants from soil. In order to test the validity of this approach, the initial objectives of the project were to study the kinetics of iodide and iodate uptake by plants from soil. Quantitative determinations of iodate in isolated soil solutions were started but due to time shortage they had to be abandoned for the time being. The experiments reported in the following were conducted in order to 1) obtain the kinetics of iodide uptake by plants from an acid soil, and 2) study if the fall in accumulation of iodine by plants with increasing additions of lime to the soil is due to changes in plant factors and/or in soil factors.

2.2. Experiment 1. The kinetics of iodide uptake by plants from soil.

The experiment was conducted in a greenhouse according to a standard pot experiment technique. Main properties of the soil are given in table 1. Treatments were: 0, 0.5, 10 and 15 ppm iodine applied as KI. There were four replicates. In one pot from each treatment soil solution was isolated by introducing a glass suction cup according to Nielsen (1972a). Six weeks after mixing basal dressing, $CaCO_3$, and KI with the soil 0.1 g seed of perennial ryegrass (Lolium perenne L. var. Vigor) was sown per pot with a capacity of 18 l of soil and a surface area of 500 cm^2. At intervals during the growth period soil solution was isolated. The pH and concentration of iodide in the soil solution were determined. The plant tops were harvested 56 days after germination. Dry matter yield and iodine concentration in dry matter were determined.

The main results are set out in figure 1 which shows iodine content accumulated in plant tops during the entire growth period as function of the mean iodide concentration in the soil solution from the 28th to the 56th day. During this time interval 90% of the total dry matter yield was produced. The final yield was independent of iodine treatment. The experimental points were fitted to the relation

$$v_m^* \Delta t = V_m^* \Delta t \frac{\bar{c}_b - c_{min}}{K_m + \bar{c}_b - c_{min}} \qquad (2).$$

v_m^* and V_m^* are the mean rate (μg $days^{-1}$) of iodide uptake and the mean maximal rate (μg $days^{-1}$) of iodide uptake respectively by aerial parts of ryegrass in one pot in the time interval Δt. For details see Nielsen (1972b, 1976). $\bar{c}_b$, c_{min}, and K_m denote the mean concentration of iodide in the bulk soil solution $\bar{c}_{b(\mu M)}$, the concentration of iodide in the bulk soil solution at which $v_m^* = 0$, and Michaelis-Menten constant for iodide uptake respectively. Values of the parameters

V_m^* Δt, K_m, c_{min}, and the pH of the isolated soil solution are given in figure 1. The values of the parameters K_m and c_{min} differ only slightly from those obtained with barley in nutrient solution culture experiments at the same pH (see Project no.1).

2.3. Experiment 2. Effect of liming a soil on the concentration of iodide and total iodine in the soil solution and iodine uptake by plants.

The experiment was conducted in a greenhouse. The pot experiment technique, the soil, and the pots used were identical with those in experiment 1. Treatments were: 0, 15, 30 and 60 g $CaCO_3$ with 0, and 1 ppm iodine, applied as KI, per pot. There were four replicates and in each treatment two plant species were grown: perennial ryegrass (Lolium perenne L.var. Vigor) and barley (Hordeum vulgare L. var. Nordal). Iodine was added to the soil three weeks before lime and basal dressing. The soil-iodine-lime-fertilizer mixture was left in the pots for three weeks. Soil samples were then taken out from each treatment and 0.1 g seed of ryegrass or 24 seeds of barley were sown per pot. After germination barley was thinned to 20 plants per pot.
In the soil samples pH in 0.01 M $CaCl_2$ suspensions and iodide extractable with a strongly basic anion exchange resin (Dowex 2 x 8, NO_3-form) were determined. Plants were harvested and soil solution isolated by displacement with 0.01 M $CaCl_2$ on columns 44 days after germination. The soil solutions were filtered through a 0.2 µm membrane filter and the total concentration of iodine as well as the concentration of iodide were determined in the filtrate.

Results of determination of pH and resin extractable iodine in soil and of iodide and total iodine concentration in soil solution are given in table 2. The concentration of iodide in the filtered soil solution constituted only a minor part of the total iodine present as organic and/or iorganic iodine. The total concentration of iodine in the soil solution did not change significantly due to liming of the soil nor did the concentration of iodide except at the largest application of $CaCO_3$. Furthermore, iodide in the soil solution constituted only about 1% of the mobile pool of iodide in the soil estimated by resin extraction. Concentration of iodine in dry matter of perennial ryegrass decreased with increasing addition of $CaCO_3$ at both levels of iodine as it is seen from table 3. By and large the iodine concentrations in dry matter of barley for various treatments were the same as corresponding concentrations in ryegrass.

2.4. Discussion and conclusion.

The relationship between iodide accumulation by aerial parts of soil grown ryegrass during the entire growth period and the mean concentration of iodide in the bulk soil solution could be expressed by an equation of the Michaelis-Menten type. The values of the parameters K_m and c_{min} were very similar to those obtained in studies on the kinetics of iodide uptake for barley plants in nutrient solution culture described in report of results in Project no. 1. The results support thus the view that the rate determining step in iodide uptake via the root system is located at the same site for iodide uptake by aerial parts of plants during a growth period as for iodide uptake by entire plants in short-time experiments provided the concentration of iodide in solution is within the concentration range studied here.

Further support of the view mentioned above is provided by results from experiment 2. In agreement with work by others, e.g. Whitehead (1975), the present results showed that increasing application of lime decreases the amount of iodine accumulated by plants from soil. Furthermore, results of experiment 2 showed that the concentration of iodide in the soil solution did not change with moderate lime applications. Total root length was not measured but changed presumably less than yield of top dry matter as a result of liming. Results in water culture experiments showed that I_n, the net uptake rate of iodide per unit of root length, decreased, even at constant iodide concentration, with increasing pH of the nutrient solution as I_{max} decreased and c_{min} increased with increasing pH of the nutrient solution (see equation 1 in Project no. 1). The obtained fall in uptake of iodine from soil with increasing application of lime is thus to be expected provided that 1) iodide is a chemical species of iodine of predominant importance in

uptake of iodine by plants from soil, 2) changes in root length with application of lime are negligible, and 3) iodide concentration in soil solution remains unaltered.

A model describing iodine and I-129 uptake by plants from soil should only incorporate parameters related to those chemical species of iodine in the soil solution which contribute significantly to iodine uptake via the root system. According to Saas and Grauby (1976) small iodinated organic molecules, present in soil solution, may, in addition to iodides, be taken up by plant roots. Present results show that reliable values of iodide uptake parameters, necessary prerequisites in predicting iodide uptake by plants, can be obtained from solution culture experiments. A study of the correlation between predicted iodine uptake, assuming that only iodide is taken up, and observed iodine uptake by plants grown in a range of different soils would further aid in clarifying the importance of iodide, relative to other chemical forms of iodine, in uptake of iodine and I-129 by plants from soil.

2.5. References.

Nielsen, Niels Erik 1972a. Plant and Soil 36, 505-520.
Nielsen, Niels Erik 1972b. Plant and Soil 37, 561-576.
Nielsen, Niels Erik 1976. Plant and Soil 45, 659-677.
Saas, A.; Grauby, A. 1976. Health Phys. 31, 21-26.
Whitehead, D.C. 1975. J. Sci. Fd. Agric. 26, 361-367.

Tabel 1. Main properties of the virgin heath sandy soil used

Mechanical fractions in %					pH	Iodine
2.0 - 0.2 mm	0.2 - 0.02 mm	0.02 - 0.002 mm	<0.002 mm	0.M. *)	($CaCl_2$)	ppm
75.7	14.7	2.1	3.1	4.4	3.9	3

*) 0.M. = organic matter.

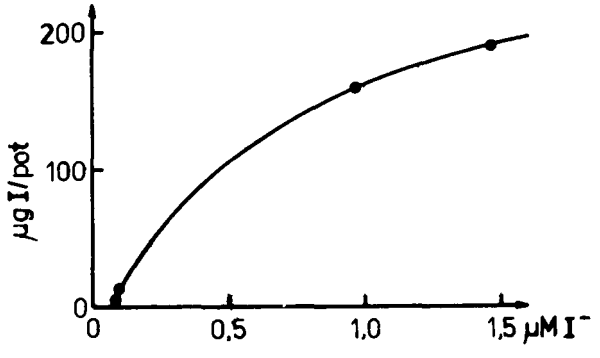

Fig. 1. Iodide accumulation ($v_m^* \Delta t$) in µg/pot in perennial ryegrass during a 56 days growth period as a function of the mean iodide concentration $\bar{c}_b$ in the soil solution from the 28th to the 56th day. (●) denotes experimental values. The curve was calculated by use of equation 2 after insertion of the parameter values $V_m^* \Delta t$ = 295 µg I/pot, K_m = 0.77 µM I^-, and c_{min} = 0.07 µM I^-. pH (soil solution) = 4.3.

Table 2. Effect of increasing addition of $CaCO_3$ to an acid soil with and without addition of iodide on the concentration of resin extractable iodide in the soil, REI, total concentration of iodine in the soil solution, C_I, and concentration of iodide in the soil solution, $[I^-]$.

$CaCO_3$ added g/pot	pH in 0.01 M $CaCl_2$	Iodide added, ppm I					
		0			1		
		REI ppm	C_I µM	$[I^-]$ µM	REI ppm	C_I µM	$[I^-]$ µM
0	3.9	0.18	0.76	0.09	0.26	1.7	0.20
15	4.5	0.25	0.90	0.12	0.24	0.9	0.19
30	5.1	0.27	0.74	0.10	0.54	1.1	0.13
60	6.1	0.30	0.74	0.22	0.57	1.3	(1.3)

Table 3. Effect of increasing addition of $CaCO_3$ to an acid soil with and without addition of iodide on dry matter yield, DM, concentration of iodine in dry matter, and uptake of iodine per pot in aerial parts of perennial ryegrass (Lolium perenne L. var. Vigor). *)

$CaCO_3$ added g/pot	Iodide added, ppm I					
	0			1		
	DM g/pot	Concentration ppm I	Uptake µg I/pot	DM g/pot	Concentration ppm I	Uptake µg I/pot
0	15 ab	0.10 a	1.5 a	12 b	0.16 a	1.9 b
15	17 a	0.07 a	1.3 a	17 a	0.16 a	3.0 a
30	16 a	0.04 b	0.6 b	17 a	0.12 a	2.0 b
60	12 b	0.02 b	0.3 b	11 b	0.04 b	0.5 c
C.V.**)	6.6	15.9	16.1	7.5	13.6	14.1

*) Figures within the same column are not significantly different if followed by same letter (P = .05).

**) $CV = 100\, s_x / (\bar{x}\sqrt{n}_{mean})$.

Contractor : Bundesgesundheitsamt
Institut für Strahlenhygiene
Neuherberg bei München.

Contract N°: 255-77-1 BIO D
Head of research team : Prof. Dr. med. F.E. STIEVE

General subject of contract : Radiation protection of workers and the general population, specifically in regard to nuclear installations.

Project results : Final Report 1977-1980

Titel of project 1 : Investigations about the distribution of Tritium and Carbon-14 and their compounds in the aqueous and organic phase of various links of aquatic and terrestric food chains.

Project director and Scientific staff :

Dr. rer. nat. G. KISTNER,

Dr.-Ing. S. STRACK,
Dr.-Ing. E. CLAUSEN,
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With the increasing use of radioactive subtances in research and technology, an enlarged knowledge in the field of radiobiological evaluation and assessment is of vital importance for decreasing the radiation risk of workers and that of the general population.

On account of the type of fixation in the various organic phases of the ecological food chains, problems concerning the effects of a possible environmental contamination with Tritium and Carbon-14 are of major importance.

An examination of the effects on biological systems is essential for the assessment of future risks. Only with sufficient knowledge of health-related consequences may a reduction of risk be effectively recognized when compared to the risks of daily life on one hand and in view of the costs involved in the technological realization on the other.

1. Organically bound tritium (literature study)
(L. Göke)

Incorporated tritium is rapidly distributed in organisms to the aqueous, and - with a certain delay- - also to the organic components, replacing the existing hydrogen (protium). Also, when administering gaseous tritium in its elementary form, tritiated water as well as more or less stable organically bound tritium will always develop in the organism. While tritiated water is quite rapidly eliminated ($T_{1/2\,biol.}$:Mice= 1.8 days, man=10 days), organically bound tritium remains in the organism for a longer period of time and, specifically, may be quite efficiently exchanged for hydrogen-atoms of the organic substance. The latter is particularly important for the metabolism of single organismus and for the transport via the food chains. While the literature contains

quite extensive reports about the influence of a tritiation on the genetic system, relatively few studies are available as, for instance, in the areas concerning the mechanismus of tritiation during the metabolic and catabolic processes, the regular retention conditions of organic tritium compounds, their reutilization after cell death and the interactions of cells and tissues in the hydrogen-metabolism. The isotopic-and transmutation effects in biological systems have not been sufficiently elucidated to correctly assess findings which indicate their significance.

2. Experimental investigations:

The following may be considered as aquatic or terrestric pathways for tritium and C-14:

HTO, $^{14}CO_2$ → phytoplankton (green algae) → zooplankton (daphnia) → fish

HTO, $^{14}CO_2$ → plant → cow → man (infant).

Thus, the experimental investigations may be limited to assessing the transition into the following structural units and their metabolic processing:

HTO, $^{14}CO_2$ → algae protein → daphnia protein → fish protein

HTO, $^{14}CO_2$ → plant protein → milk protein → human excretion

The following partial steps were examined in model trials:

2.1. Aquatic pathway

2.1.1. Kinetics of tritium incorporation in algae
(S. Strack)

Under stationary conditions, a discrimination occurs between tritium and normal hydrogen (protium) of up to 25% because of differing mass relationships. During the metabolic process, larger amounts of tritium are fixed in a manner that maintains their stable incorporation in organic molecules over weeks and months and permits their transport in the food chains in a usable form.
Under dynamic growth conditions - single injection into the growing culture - tritium is incorporated according to the pharmacologically well-known Bateman function. Accordingly, the derived values for the specific activity may be considerably higher than 1 under continuous trial conditions, if the various compartments are voided at varying rates. The particular form of concentration, occurring in natural ecologic systems as accumulation, may thus be explained. (Fig. 1 - 3).

It becomes evident that an accumulation in the various trophic levels depends on the type, frequency and timely supply of the examined substance to the system. If these parameters are known, an assessment is possible.

Repeated injections in the growing culture are followed by a cumulative adjustment of the maximum weight balance for organically bound tritium, depending on each time interval between injections and regular conditions, as they are also known for pharmaceutical maxima in blood (Fig. 4).

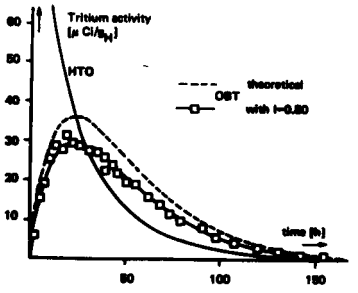
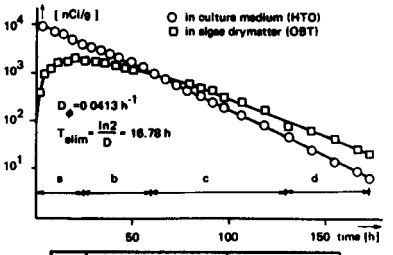


Fig. 1
Tritium concentration in a continuous culture of green algae



	D	T
a	D_1=0.0433 h^{-1}	T_1=16.01 h
b	D_2=0.0375 h^{-1}	T_2=18.48 h
c	D_3=0.0420 h^{-1}	T_3=16.50 h
d	D_4=0.0462 h^{-1}	T_4=15.0 h

Fig. 2
Tritium concentration in a continuous culture (semilog.) of green algae

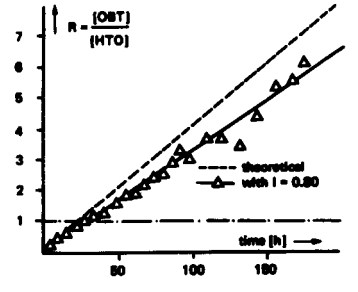


Fig. 3
R-values in a continuous culture of green algae

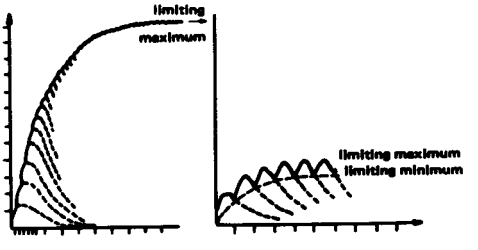


Fig. 4
Cumulative increase of organically bound tritium (OBT)

2.1.2. Kinetics of C-14 incorporation in algae
(W. Leister)

Growth conditions in stagnant waters may be simulated in batch cultures. Here, the C-14 incorporation with $NaHCO_3$ as C-source into the biomass was not equivalent to the remaining carbon. The C-14/C-12 relationship in algae logarithmically increases up to 96% from the initial value as a function of the newly formed substrate cell mass (Fig. 1). 43% of the C-14 activity is organically bound and may thus reach consecutive trophic levels.

Because of the concentration processes, the value of 0.96 is only temporary. In order to facilitate a comparison to isotopic effects when incorporating tritium in a modified batch culture, the C-14/C-12 amount was kept constant during growth by simultaneous permeation. This permitted a model of a C-14 incorporation solely in relationship to the growth function, whereby the isotopic effects of a 12% C-14 discrimination are measurable without an appreciable tendency towards decrease (Fig. 5).

Under the conditions of a dynamic C-14 exposure, the transfer from compartment to compartment at the time of increase until the C-14 activity decreased could be observed at continuous growth conditions. The model functions similarly to repeated C-14 emissions into running waters. The C-14 elimination from the medium into the bio-mass follows a kinetic of the first order. This resulted in a decreasing exponential function for the elimination from the medium and in a Bateman-function for the C-14 invasion of the biomass (Fig. 6). Respective differences in the values of the kinetic constants were evident. Following a potentially increasing course with the time of the experiment, the C-14 activity in the algae was 65 times higher at the end of the experiment than that in the medium (Fig. 7). The value of the C-14 discrimination decreased logarythmically with time to 4% (Fig. 8). One third of the C-14 activity used was organically bound. In comparison to tritium, this resulted in a 1600 times higher incorporation at the same amount of activity.

A model of permanent infusion simulated the case of a permanent C-14 emission in running waters. The adaptation phase for the C-14/C-12 relationship in algae and medium at continuous growth was examined until "quasi stationary" values were reached (Fig. 9 and 10). For this state it took about 8 1/2 doubling times. More than 1/4 of the activity used was organically bound. The C-14 discrimination assumed similar values to those of the dynamic C-14 exposure model.

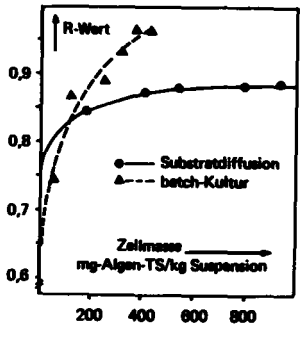


Fig. 5
Comparative demonstration of R-values as a function of the newly formed cell mass in the batch culture decreased conditions of the normal substrate, as well as that of substrate diffusion (simultaneous permeation)

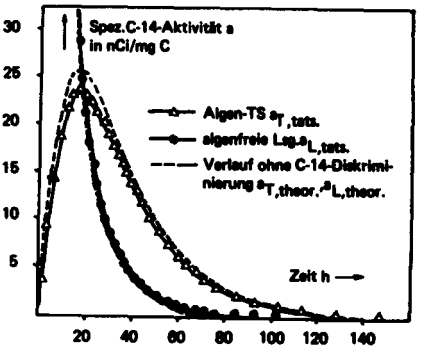


Fig. 6
Course of specific Activity (C-14/C-12-relationship) in the continuous culture after an additional single supply of C-14

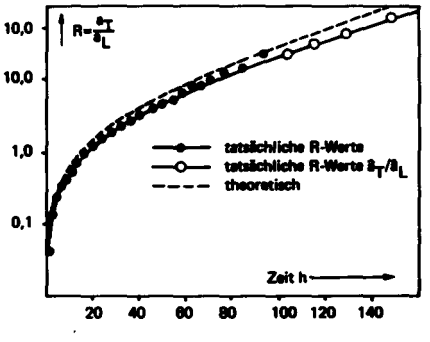


Fig. 7
R-value depending on the experimental time (the last 4 coordinates have their origin in the respective functional values

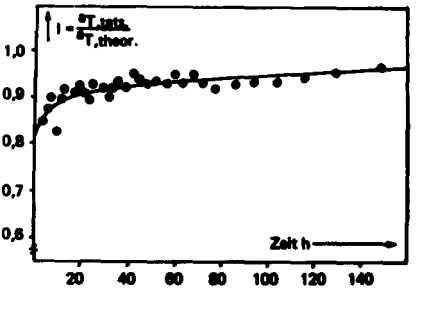


Fig. 8
Relationship I - spec. activity of algae, actual to theoretical - depending on experimental time

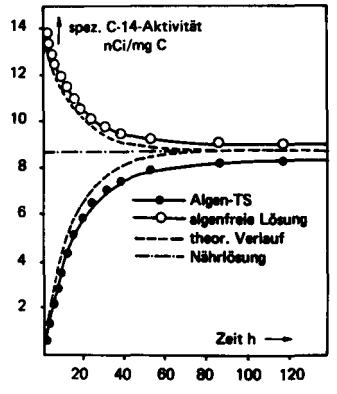


Fig. 9
Course of specific activity in the continuous culture by permanent infusion of C-14 with "priming dose"

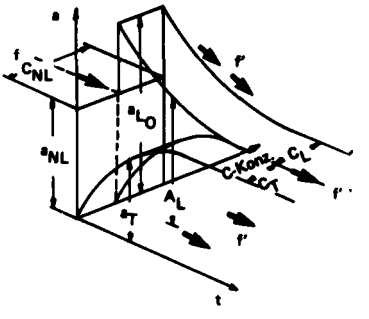


Fig. 10
Permanent infusion precedure, projected theoretically, i.e. without isotopic effects

2.1.3. Translocation of tritium from algae to Zooplankton (daphnia)
(I. Boche)

Under natural conditions, green algae are the main food supply for daphnia (small crustaceans, water fleas). The algae cells are absorbed by the daphnia by filtration and decomposed in the intestine by digestive fermentation. Proteases, for instance, effect the decomposition of algae protein down to the single amino acids which, in turn, serve the small crustacean for the build-up of its body substance.

It was demonstrated that the feeding of tritium labeled green algae leads to a measurable incorporation of tritium into the natural substance of the crustacean. Even if the food supply is large, only the amount of tritium corresponding with the existing body substance of the daphnia is incorporated.

2.1.4. Optimization of culture methods for an increased yield in the cultivation of algae
(E. Nürnberger)

The growing culture of green algae provides the basis for studies of the aquatic food chain (algae - daphnia - fish) in regard to the distribution of tritium and C-14. To assure a qualitatively and quantiatively satisfying reproduction, a modern fermentation unit was put into operation. Precisely functioning measurement - and control systems result in well reproducable growth patterns. The danger of contamination was limited

by introducing electrodes that could be sterilized, a procedure which is particularly advantageous for long term operation. The continuous pH-measurement during growth enables a good control of deviations in the carbonate-bicarbonate-relationship and thus acts as a steering mechanismus for the food supply.

The introduction of an electronic particle counter for the direct determination of cell numbers is an essential improvement. A unit designed for hematological purposes was succesfully employed for algae counting. Because of the formation of zoenobia with Scenedesmus quadricauda, counting poses a problem in the correction of coincidental losses. Counting results can be reproduced with great accuracy. The growth rate, which is an important parameter for the reproducability of the growth conditions of a culture, may now be quickly and simply estimated by correlating cell mass (dryweight) with the cell number as determined by this counter.

2.2. Terrestric pathway:

2.2.1. Tritium distribution in the structure units of proteins (E. Clausen)

Connected with investigations concerning the distribution of tritium within the terrestric transfer chain (grass-cowmilk-infant), necessary methods were developed for determining the tritium content of aminoacid components in milk protein by employing ion exchange chromatography.

According to the mode of feeding, either as tritiumwater or as tritium-labeled hay, a varying incorporation in milk casein takes place. The tritium distribution in aminoacid components achieved by acid hydrolysis is demonstrated in (Fig. 11)

Similar investigations concerning the distribution of tritium and C-14 within algae protein have been instituted in order to determine which precursory combinations might occur on the next trophic level and what might be the total balance in man after passing through the food chain.

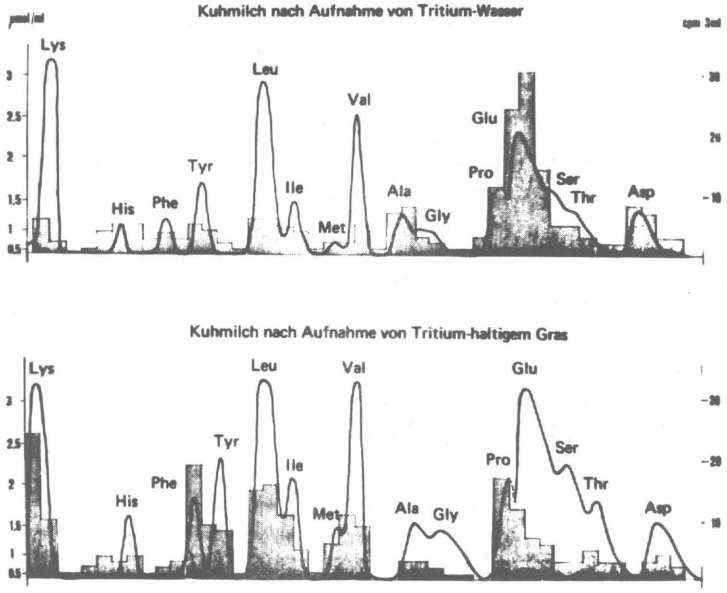
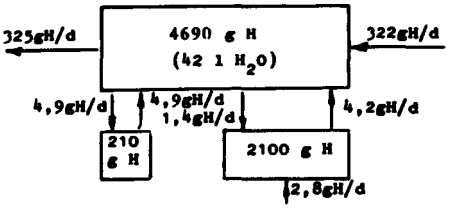


Fig. 11
Amino-acid combination and tritium in casein

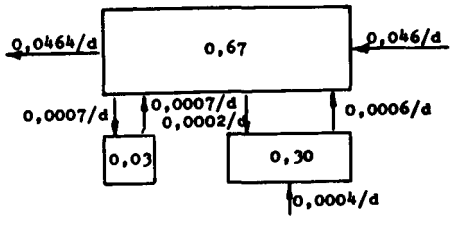
2.2.2. Assessment of radiation exposure to tritium using compartment models (E. Clausen)

On hand of mathematical models, the quantitative behaviour as to distribution and dose received has been assessed. The available hydrogen is divided into 3 compartments: 67%, which exist as water and are eliminated at a biological half life of rd. 10 days, 3% which have a biological half life of 30 days and the remainder which is more solidly bound and is exchangeable with tritium in water to a limited extent. The biological half life amounts to rd. 350 days.

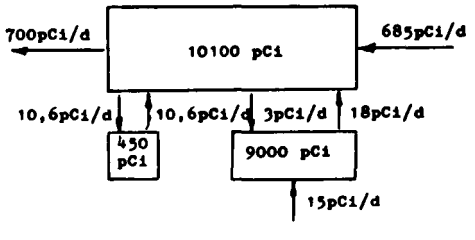
The incorporation of tritium from HTO in the organic compounds of the body is challenged by the substitution of such substances from food components. Therefore, one compartment was chosen for both and used for the assessments of long lived organic substances.



1) The total hydrogen content of the body is divided into 3 compartments. 2.8 g hydrogen per day are transferred from food into long-lived body sub stances. The remainder of the food is oxidized and channeled into the water compartment.



2) Standardized model: total hydrogen = 1



3) Example: According to Bogen (1978), the nutritional tritium concentration for the New York population amounted in 1978 to approximately 200 pCi/l water for free tritium and 600 pCi/l water for bound tritium. This results in a daily intake of 520 pCi free tritium and an additional intake of 165 pCi oxidation water. 15 pCi are channeled into the long lived pool.

Equilibrium in the organism establishes the following ratio of specific activities

$$\frac{OBT}{HTO} = \frac{\frac{9450}{0.33}}{\frac{10100}{0.67}} = \text{Ca.: } 2$$

This indicates that the specific activity in organic substances is twice as high as that in water. The result is in agreement with values observed elsewhere. Similar model assessments may also be made for other links of the food chain.

Summary

The intake and photosynthetic incorporation of tritium and C-14, administered in the form of water or C-14 O_2 to green algae, has been investigated in regard to their biokinetic patterns. It was shown that for tritium as well as for C-14, isotopic effects which are the cause of discrimination at intake and varying conversion rates of the compartments loaded with these radionuclides, are resulting in specific activity ratios that may appear as accumulation or concentration processes. The level of balance that is achieved after repeated incorporation, corresponds with pharmacologically known patterns. In additional model examinations, the size and conversion rate of compartments within the food chains will have to be determined in order to arrive at the nesessary total balance for the assessment of radiation exposure to man by tritium and C-14.

Contractor: United Kingdom Atomic Energy Authority
Harwell, Oxon. OX11 ORA, UK

Contract Nr. 187-76-1-BIO UK

Head of Research team : Dr. A.C. Chamberlain

General subject of Contract : RESUSPENSION OF PARTICLES FROM GROUND SURFACES

Title of the project nr.: RESUSPENSION OF PARTICLES FROM GROUND SURFACES

Head of project and scientific staff : Dr. A.C. Chamberlain
J.A. Garland, A.C. Wells, P.F. Muxlow

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1. Resuspension from land surfaces by the wind

Previous work indicated that resuspension could be an important source of airborne contamination in arid regions. Little work had been performed in moist temperate climates typical of North-west Europe, and this project was undertaken to determine the magnitude of resuspension in such conditions. A wind tunnel was set up on grassland or on bare soil contaminated with suitable tracers, and the variation of resuspension with wind speed, particle size, vegetation cover and moisture content of the soil was to be investigated.

The first results concerned sub-micron tungstic oxide powder, deposited by settling from the air. It was found that changes in wind speed and in the duration of weathering by the wind (Table 1, see also Garland 1979, 1981) caused most of the variation in resuspension. The resuspension rate decreased in proportion to the reciprocal of weathering time and increased with the wind speed to the third or fourth power. No consistent pattern of variation with moisture content of air or soil was apparent, and the difference between bare soil and grass 10 cm high did not exceed a factor of three.

Table 1 also compares results obtained with other particle sizes, with silt, and with $FeCl_3$ solution sprayed onto grass. Resuspension rates differed by less than a factor of 10 for a wide range of tracer materials. However, 5 μm iron oxide particles resuspended more readily than any other tracer tested, suggesting an effect of particle size on resuspension rate. The resuspension rates reported for silt from grass (Garland 1979) may be used to predict resuspension from salt marshes.

If the results are to be applied to estimate concentrations that might occur due to resuspension from extensive contaminated areas of the countryside, it will be necessary to allow for upward diffusion and deposition of the resuspended material. The importance of deposition of resuspended particles was illustrated in an experiment over grass, where 60 percent of the resuspended particles were deposited again within 4 m of travel. Measurements have been made of the deposition of particles released at various heights within an artificial grass canopy. These may be used to develop a method for calculating the rate of deposition of resuspended particles, and the concentration resulting from resuspension over extensive contaminated areas.

An experiment was undertaken to determine the effect of weathering by the natural wind and rain on resuspension rate (Garland 1981). The rate of resuspension at windspeeds of 11 to 12 m s^{-1} declined progressively during seven months from about 10^{-5} s^{-1} to 10^{-10} s^{-1}.

It was shown that the fraction of deposited material that is resuspended does not exceed a few per cent; most of the resuspension occurs within the first few hours or days after deposition, predominantly during periods when the wind speed is higher than average. It follows that the total airborne dose due to resuspension must be a small fraction of the dose which would occur from the primary cloud if no deposition occurred. The diluting effect of high winds is likely to reduce the fraction even further. It is unlikely that an airborne release which does not lead to a significant inhalation hazard from the primary plume will give rise to a significant resuspension problem later. Nonetheless the period for which control measures are necessary after an accident may be determined in part by resuspension. In this connection the results show that the potential for resuspension declines rapidly at first, and more slowly later; the time variation is better described by a reciprocal law than by an exponential one.

TABLE 1 Summary of typical resuspension rates (i.e. fraction resuspended per second) for various trace materials from grass

Type of tracer	Fraction resuspended in 1.5 h at 10 m s^{-1}	Resuspension rate, Λ, s^{-1}					
		After 10 hours weathering			After 100 hours weathering		
		3 m s^{-1}	8 m s^{-1}	10 m s^{-1}	3 m s^{-1}	8 m s^{-1}	10 m s^{-1}
Submicron WO_3 powder	10^{-2}			1.5×10^{-7}	2×10^{-10}		3×10^{-8}
$FeCl_3$ applied in solution	4.7×10^{-3}	2×10^{-9}	10^{-7}	4×10^{-7}	7×10^{-10}	8×10^{-9}	3×10^{-8}
2 μm $Fe(OH)_3$ spheres	3.6×10^{-3}	7×10^{-9}	1.7×10^{-7}	7×10^{-7}	2.4×10^{-9}	5×10^{-8}	8×10^{-8}
5 μm $Fe(OH)_3$ spheres	0.22	6×10^{-8}	8×10^{-7}	1.7×10^{-6}	8×10^{-9}	7×10^{-8}	1.6×10^{-7}
Silt applied as slurry	10^{-3}			1.2×10^{-7}			3×10^{-8}

2. Resuspension by rain splash, mechanical disturbance, etc.

Occasional observations during the wind tunnel experiments showed that water drops striking the surface, or mechanical disturbance could lead to resuspension rates a few times higher than could be attributed to the wind alone. Further work in the laboratory showed that raindrops can disperse particles from the surface of dry, or of very wet soil, and from grass blades. Resuspension in the field could include contributions due to rain splash and traffic as well as the wind. These cannot yet be quantified for recently deposited material. For deposits as old as weapons fallout (~15 years) trace element measurements show that the resuspension factor due to all causes cannot exceed $7 \times 10^{-11} m^{-1}$ on average in Britain (Garland 1979).

3. Plutonium suspension from seawater

Laboratory experiments demonstrated that plutonium is concentrated at the surface of seawater and other solutions. It was also shown that tetravalent and hexavalent plutonium is enriched in seaspray by one or two orders of magnitude (Garland 1981b). This enrichment in the laboratory suggests that a similar effect should be anticipated when deriving acceptable limits of discharge to sea.

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J.A. Garland and R.C. Chadwick, 1981. Plutonium suspension from seawater. Health Physics, to be published.

Contractor : Université Catholique de Louvain

Contract nr : 234-77-1 BIO B

General subjet of contracts : Translocation of radionuclides absorbed by the roots (redistribution) and subsequent release from leaves into the atmosphere (biological resuspension). (one project).

Title of the project nr. Translocation of radionuclides absorbed by the roots (redistribution) and subsequent release from leaves into the atmosphere (biological resuspension).

Head of Project and scientific staff : MYTTENAERE C., DELMOTTE A., HORVATH F., MOUSNY J.M., RONNEAU C., ROUCOUX P., VANDECASTEELE C., VAN HOVE C.

I. DETERMINATION OF Tc IN PLANT MATERIAL.

Preparation of samples and determination of Tc activity require a special attention due to the volatilization of some Tc chemical forms. In our experiments, contaminated plant material was dried, gently digested (HNO_3 + H_2O_2) and counted for ^{99}Tc by liquid scintillation.

- *The digestion method was tested on known contaminated living material (Azolla plants cultivated in Packard's flaks, filled with ^{99}Tc contaminated nutrient solution) : no Tc loss was observed during the preparation of the samples.*
- *A mathematical model was built to estimate the quenching percentage of Tc contaminated biological samples measured by the liquid scintillation technique (Channels ratio).*

II. DETERMINATION OF RADIONUCLIDES RELEASED FROM PLANTS INTO THE ATMOSPHERE.

1. *The volatile exudates from vegetation constitue "a priori" a way of transport of elements in the biogeochemical cycle and such a mechanism needed to be evaluated in the framework of the transfer of pollutants. Our experimentation aimed at the study of the rôle of volatile exudates in the transfer of different radionuclides absorbed by the roots and transported to the aerial parts. Different species were cultivated in nutrient solution or in soil contaminated with ^{65}Zn and ^{134}Cs. The aerial parts of fully labelled plants*

tightly isolated from the root medium were transfered to a perspex chamber ; clean dry air was passed through the chamber and the outgoing air passed through a cold finger trap ; samples were analysed for radioactivity by β spectrometry.

None of the radioactivity measurements has revealed a significant contamination of the samples collected and the results obtained have not consequently confirmed those recently published in the litterature.

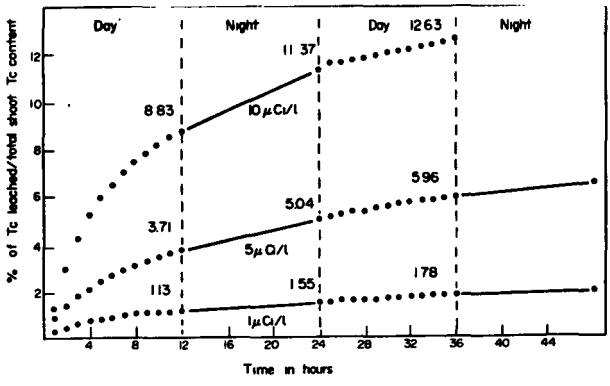
2. *The same method was applied to plants growing on a ^{99}Tc contaminated soil ; the outgoing air was passed through a finger trap containing a reducing medium :*

thiosulfate Na	*solution 1/4M*	*250 cc/l*
$Cu^{++}AcO_2^-$	*solution 1/10M*	*100 cc/l*
Dithionite Na	*solution 8g/l*	*100 cc/l*
H_2SO_4	*solution 2N*	→ *1 l*

Samples were analyzed for radioactivity by β spectrometry. First experiments revealed that very low percentage of the plant-substrate activity is detected in the trapping solution. Experiments in course will attempt to dissociate the rôle of the ecosystem components and to quantify the Tc loss.

III. DETERMINATION OF RADIONUCLIDES LEACHED OUT OF THE FOLIAGE

It has been demonstrated that organic and inorganic metabolites may be leached from foliage by rain, dew and mist and the study was designed to investigate the leachability of technetium from foliage by rain. The amount and variety of ways technetium leaching occurs may have in fact an important bearing on food contamination. The results obtained indicate that leaching may be considered as an important mechanism in the redistribution and concentration of Tc in the environment. The predominant form of Tc leached is the same as those present in nutrient solution ; nevertheless a small quantity of Tc leached is modified and some cationic forms were observed in the leachates. Moreover the more the plant is contaminated, the greater the percentage Tc leached (percentage of the activity in the aerial part).



Activity of the leachates in percent of the total activity of the aerial parts of plants grown in contaminated solutions: 10 µCi–5 µCi–1 µCi/l.

IV BEHAVIOUR AND TOXICITY OF TECHNETIUM.

IV a. Tc AND SOIL MICROFLORA.

Results obtained in our laboratory and found in the litterature seem to indicate that technetium wich is absorbed by living organism, is present in the cells partly on a more reduced chemical form than the form available (TcO_4^-). Some metabolites having a high reducing power issued from the photosynthesis, respiration and N_2 fixation process, may be responsible of such a reduction. Two microorganisms were used to study the behaviour of Tc (Rhizobium japonicum and Azotobacter chroococcum) ; the first one is only able to fix N_2 in microaerobic conditions meanwhile the second may fix N_2 in presence of O_2 . The effect of the Tc was studied in a controlled liquid medium containing or not a combined N source in order to underline the rôle of this radionuclide on the N_2 fixation. Results obtained for A.chroococcum (concentrations studied 10^{-3} to 10^{2} ppm Tc $\sim$ 10^{-5} to 1 mM or 17 nCi to 1,7 mCi/l) show that a negative effect is observed on the growth for a 1 mM concentration if the medium contains some nitrates (N_2 ase -) ; such a reduction is already observed at 1 µM without any N combined source in the medium. The last effect is associated with a higher concentration factor and a reduction of N_2 fixation. These results demonstrate clearly the rôle of the Tc on some important biological mechanisms. The concentration factors obtained for the different organisms are given in the following table I.

TABLEAU 1. - facteurs de concentration calculés sur base du poids frais des organismes ou organes pour différentes concentrations en Tc dans le milieu de culture.

ORGANISME	ORGANE ou MILIEU (N)	DUREE DU TRAITEMENT	CONCENTRATIONS EN TcO_4^- DANS LE MILIEU DE CULTURE (M)				
			10^{-8}	10^{-7}	10^{-6}	10^{-5}	10^{-4}
Soja	feuilles supér.	30 jours	490,8	291,5	242,3		
	feuilles infér.	"	360,8	276,6	145,4		
	tiges	"	19,4	22,3	19,4		
	gousses	"	22,1	16,8	16,0		
	racines	"	27,9	15,5	17,5		
	nodules	"	91,2	82,9	50,3		
	bactéroïdes	"			207,1		
A.cylindrica*	- NO_3^-	17 jours			58,6	9,4	1,9
	+ NO_3^-	"			2,9	0,9	0,7
R.japonicum	+ N organique	21 jours		5,5	2,3	1,0	0,2
A.Chroococcum	- NO_3^-	24 heures	77,6	59,5	73,8	48,9	7,2
	+ NO_3^-	"	10,6	4,2	2,8	1,6	1,0

* F.C. calculés sur base de l'estimation Poids sec = 10 % du poids frais.

IV b. ABSORPTION OF Tc by blue green algae (Anabaena cylindrica LEMM).

When A.cylindrica is cultivated in a free combined N medium, growth and N_2 fixation are reduced severely when the growing medium only contains 3,16 10^{-6} M Tc If NO_3^- is added to the medium after two days, growth is again observed and reach rapidly a normal level. Concentration factors werecalculated at the end of the experiments ; they are clearly higher in the medium wich does not contain combined N source ; Moreover these values decrease with the increase of the medium concentration. Accumulation of Tc is observed in the heterocyst cells, site of the N_2 fixation. After desorption (biological half - life of Tc is short) growth and N_2 fixation show again the normal trend.

IV c. ABSORPTION OF Tc BY SOYBEAN PLANTS.

Young Soybean plants are much more sensible to Tc than plants of 15 days. Such a phenomena which was observed for other species requires a carefull study (germination and first steps of growth) and is considered in collaboration with our colleagues of the "Unité de Morphologie et de Cytologie Végétales". The Tc transfer factors observed in controlled conditions are 20 times lower in roots than in leaves but the Tc is 3.5 times more concentrated in the nodules than in the rooting system (table 1). The analysis of the plant sap shows that Tc is mostly on the TcO_4^- form, which explains its very high mobility in the plant. In the leaves, according to the reducing power of the cell, most of the Tc ($\pm$ 80%) is bound to some molecules of MW wich range from 4000 to 10000. A large fraction of the Tc fixed in the roots is found in the bacteroids. Present in the solution as TcO_4^- ; this element is transfered through the cell membrane by active transport (energy requiring process).Notwithstanding the fact that Cd induces the formation of sulfur amino acids (which can reduce the TcO_4^- and fix it in the cellular medium), Cd incorporated before any Tc contamination of the medium, reduce the absorption of this element ; the reduction is a function of the Cd content of the plant. These results ask for more work in the field of the stability of the complexes formed by Tc or Cd and some metabolites.

V. TRANSFER OF Tc FORM SOIL TO PLANT.

Plants of Pisum sativum (var. Merveille de Kelvedon) were grown on seven typical european soils contaminated with different levels of ^{99}Tc (0,17 ; 1,7 and 17 µCi/Kg). Added initially as pertechnetate, the technetium absorption has been studied for three successive cultures. The translocation of technetium from soil to plant leaves is high, but its transfer is reduced in soils rich in organic matter (Fen) or poorly drained (Braunerde). Aging reduces the technetium transfer and modifies its relative distribution in plant (relatively more technetium is found in fruits) ; these results let suppose some modification of the technetium chemical form in soils with time. (table 2).

Table 2. Concentration factors for leaves (cpm per gr dry matter/cpm per gr dry soil).
(Mean ± standard error).

Soils	concentration level in ppm	Cultures		
		I	II	III
Alluv. Gley	0.01	198 ± 12	104 ± 8	84 ± 8
	0.1	192 ± 35	109 ± 10	105 ± 27
	1	408 ± 12	217 ± 5	242 ± 6
Pseudogley	0.01	267 ± 10	139 ± 12	87 ± 10
	0.1	371 ± 20	156 ± 19	140 ± 20
	1	446 ± 45	166 ± 29	138 ± 28
Braunerde	0.01	73 ± 62	30 ± 20	23 ± 15
	0.1	53 ± 25	28 ± 16	21 ± 12
	1	172 ± 40	71 ± 30	57 ± 21
Terre fusca	0.01	216 ± 18	127 ± 2	100 ± 13
	0.1	249 ± 20	141 ± 8	94 ± 8
	1	232 ± 17	159 ± 4	109 ± 16
Rendsina	0.01	203 ± 33	119 ± 7	78 ± 7
	0.1	201 ± 34	124 ± 3	93 ± 7
	1	206 ± 35	126 ± 10	77 ± 11
Podsol	0.01	246 ± 30	107 ± 7	66 ± 7
	0.1	305 ± 21	117 ± 10	77 ± 7
	1	273 ± 34	135 ± 2	91 ± 8
Fen	0.01	118 ± 6	74 ± 8	58 ± 6
	0.1	128 ± 12	75 ± 7	55 ± 8
	1	141 ± 5	78 ± 5	67 ± 2

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Contractant van de Commissie: Rijksuniversiteit Gent
Nummer van het contract: 275-79-1 BIO B
Hoofd van het researchteam: Prof. Dr. A.J. Deruytter
Algemeen onderwerp van het contract : Measurement and analysis of the evolution of the ^{85}Kr activity in the atmosphere, and study of the synergistic effect of ^{85}Kr and chemical pollution.

Titel van het project nr 1 : Measurement and analysis of atmospheric ^{85}Kr-activity.
Projectleider en wetenschappelijke medewerkers : Prof. Dr.A.J. Deruytter Dr.G.Eggermont, Dr.A.Janssens, F.Raes, Dr.E.Cottens.

The objectives of this project are the monitoring of the ^{85}Kr concentration in the atmosphere on the one hand, and the evaluation of the potentiality of observable effects of low-level radiation on atmospheric aerosols on the other hand. The project was set up in the light of the expected growth of nuclear power in the next decades, and continuation of the total release of ^{85}Kr in the reprocessing plants. This irreversible pollution could yield a significant β-radiation skin dose to the world population, and secondly an effect on the atmospheric aerosol could be expected from the measurements performed by Vohra (Vohra,K.G.,Symposium IAEA-SM-181 (1974),109). Thus it was thought to be necessary to start in time the systematic monitoring of the ^{85}Kr-concentration and the evaluation of its effect,to support the radiation protection policy in this matter. These objectives have fully been met by the results of the work performed during the two years of the contract (1979-1980).

SAMPLING AND COUNTING METHOD

A technique for the chromatographic separation of Kr has been developed (Ref.1) allowing a recovery of more than 50%. About 1 m^3 of air is sucked to and absorbed on a cooled activated charcoal trap during 1 h, traversing cooling traps for H_2O and CO_2 removal(immersion in liquid nitrogen is applied for any cooling). Then the charcoal trap is heated and purged with helium, for transfer of the gases to a cooled 5Å molecular sieve (MS). After removal of the cooler, the MS is eluted in a helium carrier flow to a thermal conductivity detector, monitoring the sequence of gases. Short after the observation of the Kr-signal, this fraction is directed to a second MS-trap, the residual gases being vented. This proce-

dure is repeated two times, thus augmenting the purity. It is found to be necessary to standardize the switching of the valves to eliminate the sporadic presence of residual N_2-gas.

Helium is pumped off from the last cooled trap, but there remains a residual pressure which has to be corrected for, in order to determine the mass of the sampled Kr-gas. This residual pressure has been determined very accurately by mixing a known mass and activity of Kr in the helium stream (Ref.4). The Kr-gas is then condensed in a scintillation vial through a glass conduct with a narrowing which is fused afterwards. The scintillator liquid (Insta**fluor**) is injected through a Teflon-coated septum under the screw cap of the vial. Thus we work with a closed system,and the Kr mass is determined very accurately. The ^{85}Kr-activity in the vial is counted, and the atmospheric concentration is derived through the known amount of Kr in air.

MEASUREMENTS AND DISCUSSION

In the course of the years 1979 and 1980 a large number of atmospheric measurements have been performed, which are reported on Fig.1.

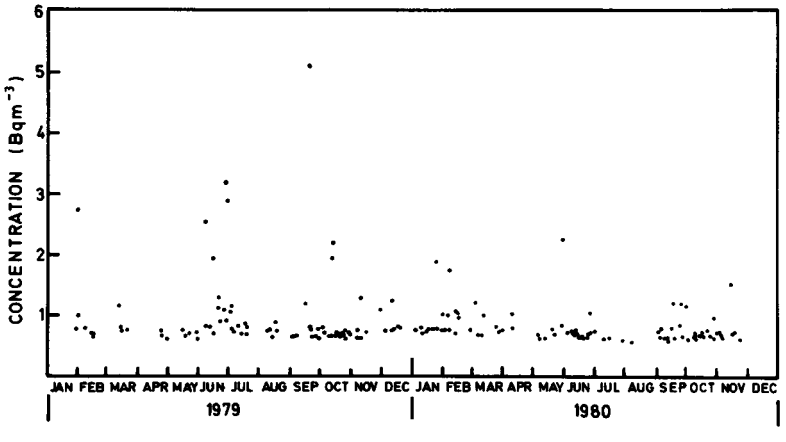

Fig.1.: ^{85}Kr-activities measured from January 1979 to December 1980

Is is noteworthy that the background activity seems to be constant, although in the period 1970-1980 an increase of the concentration from about 0.5 Bq m^{-3} to 0.7 Bq m^{-3} has occurred, due to the world reprocessing activities.

A number of peak concentrations have been measured, mainly

in June and from September to February (see Fig.1). In regard of the predominant S-W wind direction during measurement of the increased activities, their origin was promptly situated as the reprocessing centre of La Hague, France. This hypothesis has been verified. First, calculations of the trajectories have been performed, from synoptic data in 22 stations, and puf-model calculations with gaussian dispersion of the plume (Doury,A. et al., Rapport DSN 84) have been applied to a hypothetical release (3000 Ci in 3 h, official data being withheld). All the calculations show that the trajectories pass sufficiently close to Ghent to yield a concentration of at least 10 pCi m^{-3}(0.37 Bq m^{-3}). A programme is elaborated for drawing contourlines on the map for given concentration levels, and for plotting the time profile of the concentration in Ghent (Ref.2).

Secondly a statistical analysis of the frequency of the peak concentrations has been performed. A Monte-Carlo simulation is used, based on the distribution of wind directions and velocities in Mol,Belgium, and on a hypothetical release pattern, corresponding to an average release of 2500 Ci/day. The frequency of samples with an activity larger than 0.2 Bq m^{-3} is of the order of 5%, which is in rather good agreement with the actually observed picture.

EFFECT OF U.V. AND IONIZING RADIATION ON FORMATION AND EVOLUTION OF AEROSOLS.

It is known that U.V.-illumination of air containing SO_2 and NO_2 causes through the formation of OH radicals the oxidation of SO_2 to SO_3. After reaction with H_2O, sulphuric acid is formed which nucleates to form tiny droplets (aerosol). It is presumed that synergism of U.V. and ionizing radiation can affect the formation of aerosols through an enhanced transformation of SO_2 to H_2SO_4 and through an accelerated nucleation in the presence of ions. To study these effects a flow reactor has been built.The NO_2 and SO_2 concentration in pure air and the humidity are controlled.The gas passes through a 40 cm long quartz tube for illumination with U.V. (1500 μWcm^{-2}) and with a Co-60 γ source (250 $\mu Gy\ h^{-1}$). The flow-rate is variable and determines the passage time. Measurement of the particle concentration is performed with a TSI continuous condensation nuclei counter.

The flow reactor thus allows to measure the evolution of the particle concentration as a function of (passage) time. In Fig.2 this function has been plotted for illumination with U.V. on the one hand, and for simultaneous irradiation with Co-60 on the other hand. The ratio of

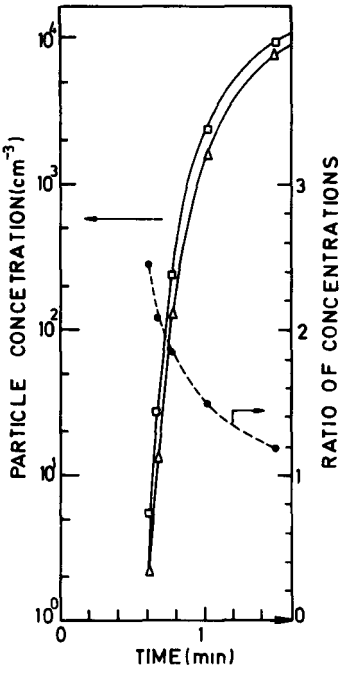

Fig.2.:
Particle concentration as a function of passage time, for air containing 0.15 ppm SO_2 and 0.15 ppm NO_2, at 0% R.H, for illumination with U.V. only (Δ) and simultaneous exposure to γ-radiation (□). The ratio of the latter to the former is given as a dashed line with the ordinate on the right side of the figure.

the latter to the former decreases from about a factor 3 to unity for longer passage times. There is thus a pronounced effect in the nucleation phase. The effect in the condensation phase will be studied in the future with use of particle distribution measurements and chemical analysis.

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2. Van Peteghem D., Studie en meting van de pollutie door Krypton-85, Thesis University of Ghent , 1980, 66 p.
3. Nuclear Physics Laboratory, Annual Report 1979, 63-67
4. Nuclear Physics Laboratory, Annual Report 1980, in print.

III. 3

GENETISCHE WIRKUNGEN IONISIERENDER STRAHLEN

HEREDITARY EFFECTS OF IONIZING RADIATIONS

EFFETS HEREDITAIRES DES RAYONNEMENTS IONISANTS

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Tätigkeitsbericht beschrieben :

Further research work on these subjects will also be described in the following progress reports :

D'autres travaux sur ce thème de recherche sont également décrits dans les rapports suivants :

185-BIA N	ITAL, Wageningen (de Zeeuw/Ringoet)
232-BIO B	CEN, Mol (Maisin)
101-PST I	ENEL, Torino (Farulla)
099-PSA F	CEA, Fontenay-aux-Roses (Uzzan)

Contractor:	University of Aarhus
Contract No.:	204-76-1 BIO DK
Head of Research Team:	O. Westergaard
General Subject of Contract:	Studies of the interaction between DNA repair and replication in the eukaryotic organism Tetrahymena pyriformis

Title of the Project:	Molecular events of ionizing radiation on specific genes
Head of Project and Scientific Staff:	B. Bonven, S. Borchsenius, B. Borkhardt, M. Carin, E. Gocke, C. Herskind, B. Grabowska, D. Jentsch, J. C. Leer, K. A. Marcker, O. F. Nielsen, D. Tiryaki, O. Westergaard

Understanding of the effect of ionizing radiation on the genetic material requires detailed knowledge of the action of radiation and of the DNA repair process at the molecular level of chromatin. In order to obtain such detailed information we have studied the effect of radiation and repair on the molecular level of a single gene:

A. Isolation of a single gene in its functional chromatin form and the effect of ionizing radiation on isolated material

The genes coding for the ribosomal rRNA precursor in Tetrahymena exist as small extra chromosomal genes. This has allowed us to isolate the gene in its functional chromatin form (3, 6, 7). The structure of the extra-chromosomal DNA is given on figure 1. The DNA exists as a giant palindrome consisting of two genes.

The DNA molecule is a replicative unit with the origin of replication near the middle of the molecule. It contains two transcriptional units, as demonstrated on the map. Each unit has an intervening sequence with a size of 400 bases in 25S region of the gene (21).

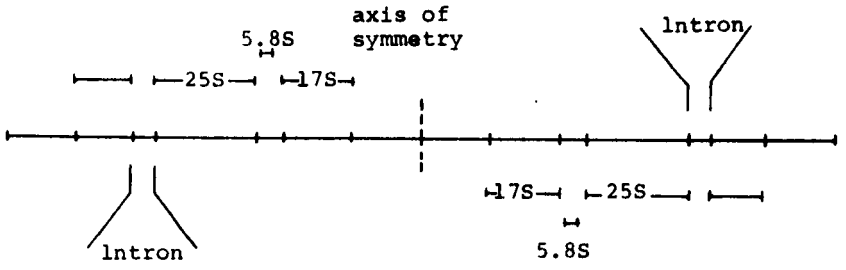

Figure 1.

We have developed techniques which allow isolation of the ribosomal gene in its functional chromatin form. Studies of the isolated chromatin has revealed the following properties (9, 12, 3): 1) The chromatin structure of the active region differs from the inactive region on the DNA (18). 2) The endogenous RNA polymerases on the active region of gene transcribe the gene faithfully in vitro (14). 3) The process of transcription termination can be regulated in vitro (15, 16, 20). Thus, the polymerases can transcribe both the active and the inactive region on the DNA. 4) The processing (splicing) of the intervening sequence in the transcript can occur on the level of the isolated chromatin (19, 21).

As the isolated chromatin mimics the in vitro situation, it has been used for studies of the effect of radiation on the molecular level. Three different approaches have been undertaken in order to study the radiation damage:

a) Techniques have been developed which allow visualization of the exact position of single strand breaks on the gene by electron microscopic studies. The technique takes advantage of the fact that the isolated chromatin is a giant palindrome and that it therefore can form snap back molecules. The technique has made it possible to compare the relative radiosensitivity of (i) the gene in vivo, (ii) the isolated gene in its biologically active chromatin form and finally, (iii) on the level of the naked DNA (22, 17).

b) Radiation induced damages can also be monitored by the endogenous RNA polymerases on both the active and the inactive regions as the signal for transcription termination can be controlled in vitro. Using the different fidelity of the RNA polymerase observed in the presence of different divalent ions we are able to monitor different types of radiation damages

on the gene. Both direct and indirect radiation effects have been monitored on the chromatin. Also a number of radioprotective agents and sensitizers have been tested on the system.

c) Digestion experiments with various nucleases have demonstrated three hypersensitive sites on the isolated chromatin. One sensitive site is located at or very near the origin of replication, while the remainins two sites are located at the promotors. Experiments are now in progress on a further characterization of these sites in relation to the functional activity of the gene. In addition it is investigated if nuclease experiments can be used to monitor radiation induced lesions and repair on the level of the DNA (1, 2, 4). In conclusion, the three described methods makes it possible to develope sensitive and precise in vitro assays for radiation induced lesions (5, 22).

B. Structure and function of genes coding for radiation induced DNA polymerases

Irradiation of exponentially growing cultures of Tetrahymena with electrons induced a DNA polymerase up to 50 fold in the cells. The polymerase is located in the mitochondria but encoded for by the nuclear genome. The induced polymerase has been purified and specific monoclonal antibodies are beeing raised against the enzyme. The synthesized monoclonal antibodies will allow a detailed study of the cellular localization of the enzyme under different conditions. In addition the synthesized antibodies will make it possible to characterize and study the structure and the function of the gene(s) coding for induced repair polymerases (5, 8).

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Contractor: Aarhus University, Department of Chemistry

Contract nr: 262-78-1 DK

Head of the research team(s): Julio E. Celis

General subject of contracts: "Search for the biochemical events leading to the lethality of mammalian cells irradiated with X-rays".

Title of the project: Search for the biochemical events leading to the lethality of mammalian cells irradiated with X-rays.

Head of project and scientific staff: J.E. Celis, R. Bravo, A. Celis, J. Bellatin and S.J. Fey.

The aim of this contract was to search for sensitive assays to determine radiation damage at the level of polypeptide synthesis, protein-nucleic acid interactions and cell cytoarchitecture.

Summary

Our studies in human HeLa cells have revealed a nuclear (IEF 49, MW 36K) and a cytoplasmic polypeptide (IEF 52, MW 34K) whose relative proportions are sensitive to changes in the growth rate produced by lethal doses of X-rays. Given the common occurance of IEF 49 and 52 in most human, hamster, mouse and rat cell lines studied and their coherent behaviour in response to different conditions under which the rate of cell proliferation declines, it is suggested that these proteins may play important if not key roles in cell proliferation. These polypeptides represent potential markers for determining radiation damage at the level of gene expression.

We did not find any significant protein-DNA crosslinking or damage to cyto-architectural components as a result of X-ray irradiation, at least to the limit of sensitivity of the methods used.

Results

Identification of two polypeptides whose rate of synthesis are sensitive to changes in growth rate caused by X-ray irradiation

During the tenure of this contract we have carried out a detailed study of the polypeptides synthesized by human HeLa cells irradiated with X-rays in an effort to search for the biochemical event leading to cell death. Using newly developed labelling techniques and high resolution two dimensional gel electrophoresis

(refs 1,4 and 7) it has been possible to separate and catalogue 1169 polypeptides (855 acidic and 314 basic) from as few as 100 control HeLa cells labelled with [^{35}S]-methionine (Fig. 1).

A comparison of the [^{35}S]-methionine labelled polypeptides synthesized by active HeLa cells and giant cells produced by irradiation with lethal doses of X-rays (1100 rads) revealed a nuclear (IEF 49; MW 36K; Fig. 1) and a cytoplasmic polypeptide (IEF 52; MW 34K; Fig. 1) whose relative proportions (rate of synthesis) correlate well with the proliferating state of the cells. The relative proportion of IEF 49 is high in proliferating control cells but is 5-6 fold lower in non-dividing irradiated cells. Conversely, the relative proportion of IEF 52 increases following irradiation. Studies of other physiological conditions in which there is a decrease in the rate of cell proliferation (ageing for example) also led to the same results. The relative proportion of IEF 49 has also been shown to change in S phase of HeLa cells (ref. 3) and during transformation of mouse and hamster fibroblasts (ref. 5).

Irradiation of HeLa cells with lethal doses of X-rays does not result in crosslinking of IEF 49 to DNA.

To determine whether cell death was due to crosslinking of proteins to DNA we irradiated prelabelled HeLa cells with different doses of X-rays and analyzed their polypeptide patterns by means of high resolution two dimensional gel electrophoresis and quantitated the major spots. Crosslinked polypeptides were expected to remain at the origin of the first dimension. Quantitation of 150 major polypeptides including many nuclear and cytoarchitectural polypeptides failed to reveal significant crosslinking of any of the studied proteins (Fig. 1) (ref. 8).

Conclusions

Our studies revealed polypeptides that may be involved in controlling cell proliferation and that constitute usefull markers in assessing radiation damage at the level of polypeptide synthesis. Whether radiation affects directly or indirectly the synthesis of these proteins remains to be elucidated

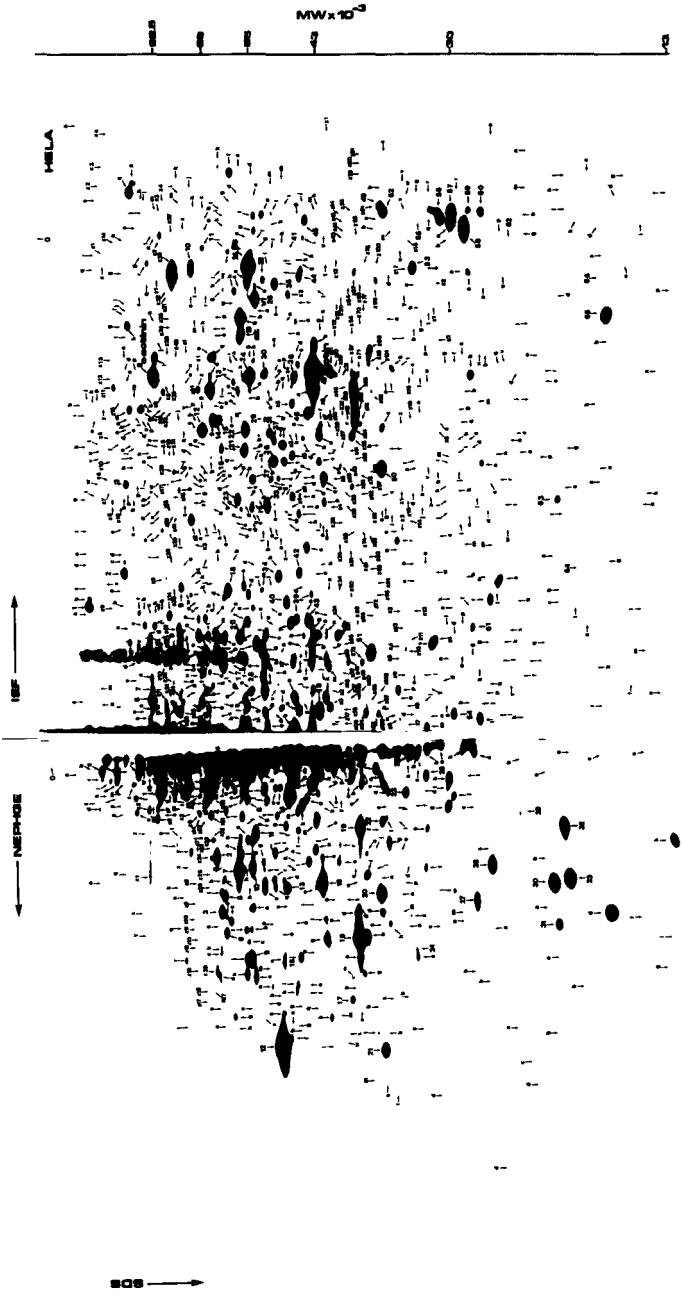

Figure 1. Separation of 1169 [^{35}S]-methionine labelled polypeptides from human HeLa cells by means of high resolution two dimensional gel electrophoresis (IEF and NEPHGE). A total of 73 nuclear and 59 cytoplasmic polypeptides have been identified (ref. 8). Many polypeptides have been identified as cytoskeletal or soluble (in preparation).

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10. Identification of a nuclear and of a cytoplasmic polypeptide whose relative proportions are sensitive to change in the rate of cell proliferation. Bravo, R., Fey, S.J., Bellatin, J., Mose Larsen, P., Arevalo, J. and Celis, J.E. Proc. Natl. Acad. Sci., in press

Contractant of the Commission:	State University of Leiden
Contract Number	: 194-76-1 BIO N
Head of the Research Teams	: Prof.Dr. A. Rörsch and Dr. P. van de Putte
General Subject	: Molecular Aspects of Repair Phenomena in Bacteria.

Title of Project Nr. 1	: The excision repair process: its genes, enzymes and regulation.
Head of Project and Scientific Staff	: Dr. P. van de Putte, Dr. H. Pannekoek, Drs. E.A. Poot-van den Berg, Dr. C.A. van Sluis, Drs. J.A. Brandsma

Organisms have the capacity to eliminate damaged segments of their genetic material (DNA) which can arise upon irradiation with UV-light. In the bacterium Escherichia coli, and possibly in other organisms as well, the excision repair process constitutes a major pathway for maintenance of the integrity of the DNA. Due to this process, pyrimidine dimers, induced by UV-irradiation, are efficiently removed and substituted by normally basepaired residues. The initial and most crucial step is the recognition of this specific lesion, followed by introduction of a single-stranded break adjacent to the lesion. This reaction is catalyzed by an enzyme-complex, composed of three different polypeptides, i.e. the UvrA, UvrB and UvrC proteins. Our goals are:

i) to study the mechanism of action of the UvrA-UvrB-UvrC enzyme-complex,

ii) to study the regulation of expression of the three uvr genes which encode the proteins involved in this so-called incision-reaction.

Although the basic principles of the excision repair process have been known for several years, no specific uvr gene product had been identified, nor had anything been learned about the regulatory elements controlling the uvr genes. Studies had been hampered by the extreme low amounts of the incision enzyme in the cell and by the rapid dissociation of the enzyme-complex upon purification with chromatografic techniques.

The development of recombinant DNA technology enabled us to overcome these drawbacks. At present, we have constructed recombinant plasmids which carry the $uvrA^+$ gene (pJA-series), or the $uvrB^+$ gene (pNP-series) or the $uvrC^+$ gene (pCA-series). During the contract period these plasmids have greatly facilitated our investigations concerning the structure, function and regulation of the uvr genes.

For the isolation of the uvr genes we have employed either transducing phages ($\lambda uvrB^+$) or the E.coli 'colony bank'. The colony bank is a collection of about 2,000 strains harbouring different plasmids (colE1-derivatives; pLC-series) carrying each a random fragment of the E.coli genome.

Mobilization of these colony bank plasmids to an uvr^- recipient strain showed that pLC25-23 contains the $uvrB^+$ gene, whereas pLC13-12 and pLC 7-18 carry the $uvrC^+$ gene. The collection of strains does not contain a plasmid harbouring an intact $uvrA^+$ gene.

We developed a novel procedure to clone genes (or mutant genes), located on the chromosome, for which no direct selection is available. The method is based on homologous recombination in a $recA^+$ $polA_{ts}$ strain at non-permissive temperature between a colony-bank plasmid, carrying a bacterial segment originated from a chromosomal site adjacent to the gene to be cloned, and the chromosome. The bacterial segment was made to contain an easy selectable marker (e.g. transposon Tn1 (Ap^r)), resulting in a high co-transduction frequency of Ap^r and the desired gene. This procedure was successfully applied to the cloning of the $uvrA^+$ gene by a partial digestion of total E.coli chromosomal DNA with SalI and subsequent insertion of an $uvrA^+/Ap^r$-containing SalI fragment on a multicopy plasmid. The resulting plasmid, pJA01, was shown to harbour the $ubiA^+$, $lexA^+$, ssb^+ and $uvrA^+$ genes. Derivatives (pJA02, pJA05 and pJA08) were constructed lacking all of these genes, except for the $uvrA^+$ gene. Complementation of the Uv^r phenotype of $uvrA^-$ strains with pJA-plasmids has been carried out and the location and orientation of the $uvrA^+$ gene on the plasmids has been established.

Molecular cloning of the $uvrB^+$ gene has provided us with many data concerning its complicated regulation and possibly dual function of its gene product. The $uvrB^+$ gene has been cloned both by EcoRI-digestion of a transducing phage ($\lambda b2att^2$ $bioFCD^+uvrB^+$) and by PstI or PstI/BamHI-digestion of the colony-bank plasmid pLC25-23. The 'phage derived' recombinant plasmid, i.e. pNP5, was shown to carry the C-terminal part of the $uvrB^+$ gene fused to an effective promotor of the vector pMB9 of this plasmid. In the presence of either one of the non-sense suppressors supF and sup6 plasmid pNP5 was found to fully complement the UV-sensitivity of ΔuvrB strains. Moreover, the incision activity in extracts of ΔuvrB/pNP5 strains was restored to the level of wild-type $uvrB^+$ strains. We conclude that in an appropriate genetic background (sup6/supF ΔuvrB) plasmid pNP5 encodes a truncated UvrB protein (MW 62,000 dalton), lacking an N-terminal fragment, which has enzymatic properties in the incision reaction equivalent to the intact UvrB protein (MW 80,000 dalton). Hence, the C-terminal part of the protein is involved in determining UV-resistance, in concert with the UvrA and UvrC proteins, whereas the N-terminal part is implicated in regulation as indicated by preliminary experiments. This conclusion is further strengthened by the observation that the N-terminal part of the uvrB gene can be mutated without loss of its enzymatic function. Evidence has been derived from the results of a procedure, developed in this laboratory, to relieve the polar effect of a transposon (Tn5 (Km^r)), integrated in the cloned uvrB gene. This procedure consists of an in vitro elimination of a major part of Tn5 and a subsequent in vivo propagation of the resulting products, yielding recombinant uvrB-plasmids with a site-specific non-polar mutation. We have demonstrated that a non-polar mutation in the N-terminal part of the gene does not affect the enzymatic function of the UvrB protein. Other relevant data, concerning the $uvrB^+$ gene, obtained with plasmid pNP5 are e.g. the establishment of the orientation (both on the plasmids and on the chromosome), the size of the gene and, moreover, a determination of the localization and the DNA-sequence of the pMB9 promotor required for the expression of the cloned $uvrB^+$ gene.

The 'colony-bank derived' uvrB$^+$-recombinant plasmids pNP10 (= pBR322-uvrB$^+$) and pNP12 (= pACYC177-uvrB$^+$) were shown to contain the entire uvrB$^+$ gene, including its transcriptional- and translational regulatory elements. The position of the elements (i.e. promotor, initiator codon) has been determined by DNA-sequence analysis of a restriction fragment, comprising the entire uvrB$^+$ regulatory region and a N-terminal part of the structural gene. A comparison of the proteins encoded by pNP10 (pNP12) DNA with 'mutant' uvrB plasmids (e.g. pNP10::Tn5), allowed us to identify the uvrB$^+$ gene product to be a protein with a MW of about 80,000 dalton. Currently, this protein is purified to study in vitro both its regulatory and its enzymatic function.

Cloning of the uvrC$^+$ gene has been accomplished using a PstI-fragment, derived from the colony-bank plasmid pLC13-12, which has been inserted into several multicopy plasmids (i.e. pCA32 (= pBR322-uvrC$^+$), pCA79 (=pACYC177-uvrC$^+$)). These uvrC$^+$-recombinant plasmids fully complement the UV-sensitivity of uvrC$^-$ strains and, moreover, restore the incision activity on UV-irradiated DNA in extracts of uvrC$^-$/pCA32 strains. We have shown both in a 'mini-cell-' and in a 'maxi-cell system' that the uvrC$^+$ gene encodes a protein with a MW of about 28,000 dalton. Employing the results of a detailed restriction enzyme analysis of plasmid pCA32, numerous 'mutant' uvrC derivatives have been constructed which enabled us to establish both the localization and the orientation of the uvrC$^+$ gene on plasmid pCA32.

Recently, we have reported on another function of the UvrC protein, besides its enzymatic role in the UvrA-UvrB-UvrC complex. In a maxi-cell system, containing compatible recombinant plasmids carrying the uvrB$^+$ and the uvrC$^+$ genes, evidence has been obtained that the UvrC protein reduces the biosynthesis of the UvrB protein. This effect is exerted at the level of initiation of transcription of the uvrB$^+$ gene. We have established that the site of interaction of the UvrC protein is located within the regulatory region of the uvrB$^+$ gene. In view of the observation, made by another laboratory, that the uvrA$^+$ gene is controlled by the lexA-recA system, we conclude that the expression of the E.coli uvr genes is strictly regulated, while different repair pathways (excision, recombination, SOS) are intimately connected as far as their regulation is concerned.

The results obtained by our laboratory during the past contractperiod and the contribution of two other laboratories (Dep. of Therapeutic Radiobiology, Yale University, New Haven, U.S.A. and the Norwegian Defense Research Establishment, Kjeller, Norway) has provided valuable information concerning structure, function and regulation of genes essential for the excision repair process. Now that all three uvr genes have been cloned on multicopy plasmids, it is conceivable that the gene products will soon be purified and available for biochemical studies. It is anticipated that the main questions, concerning the regulation and the mechanism of action of the UvrA-UvrB-UvrC complex on UV-irradiated DNA will be clarified in the next 4 years. Finally, we support the notion that the data with the model organism Escherichia coli are valuable information for understanding corresponding processes in other organisms, such as humans.

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Title of Project Nr 2	: The mechanism of mutation induction and its relation to cellular repair processes.
Head of Project and Scientific Staff	: Dr. P. van de Putte and Dr. B.W. Glickman. Drs. J. Brouwer, P. Todd and Ir. M.R.M. Schaaper.

The object of this research project is to determine the molecular mechanisms underlying mutagenesis and to evaluate the role of cellular repair processes in determining the fate of chemically induced premutagenic lesions. The research of the last 5 years has been aimed at the study of mismatch repair, the influence of mutators on mutation induction and on the mutation specificity of mutagenic agents.

1. Mismatch repair

It was shown that DNA-methylation at position 6 of adenine residues in DNA, which is defective in a Dam$^-$ mutant, plays a role in post-replication error avoidance in E.coli. (1). Dam$^-$ mutants exhibit a 20 x enhanced spontanous mutation frequency, mainly due to a strong stimulation (140 x) of GC → AT transition events. (2). Furthermore second-site revertants of Dam$^-$ mutants were isolated by selection on resistance against 2 aminopurine. These mutants, which all map on the known mutator loci mut H, mut L and mut S, show mutator activity on their own, when separated from the dam mutation and all exhibit a hyperrecombination phenotype. Experiments with heteroduplex λ-DNA, which contains a methylated- and a non-methylated strand, showed a preferential loss of markers on the non-methylated strand, after infection of a E.coli host with mut H, L or S mutation. (3). Screening of the spontanous mutation frequencies for four different markers revealed that the mutator activity of the dam mutation is different from that of the mutation enhancing plasmid pKM-101. (4).

2. R46-pKM101

Using a system which allows partitioning of arg$^+$ revertants of E.coli AB1157 into four classes, it was shownthat plasmid R-46, from which pKM101 is a derivative, alters the mutational specificity of MMS in E.coli. (5). Furthermore it appeared that protection against UV-irradiation by pKM101 is dependent on the UvrE and Recl functions, whereas plasmid enhanced UV mutagenesis is not. (6).

3. Mutagenic agents

a) Alkylating agents

A few years ago, work in our laboratory revealed the existence of a repair pathway for the removal of bases that had been misincorporated during DNA replication. This 'methylation instructed error avoidance process' involves the recognition and excision of mismatched bases and differentiated between the 'correct' parental- and error containing daughter- DNA strands on the basis of the methylation level of the

DNA. Parental DNA strands are fully methylated whereas daughter strands, following DNA-replication are not or incompletely methylated. Dam⁻ mutants of E.coli are defective in DNA-methylation and therefore discrimination between the DNA-strands is impossible in these cells. As was shown earlier the alkylating agent MMS is not capable to induce mutants in cells of a Dam⁻ strain, whereas it does induce mutants in w.t. cells.
This study has been extended to other alkylating agents i.e. EMS, MNU, ENU and MNNG. All these agents do induce mutants in w.t. cells, although with various efficiencies. We showed that MMS, MNU and MNNG, in contrast to EMS and ENU, do not induce mutants in cells of a Dam⁻ strain. This suggests that a mismatch correction mechanism, which is impaired in a Dam⁻ strain, is essential for the fixation of mutations induced by methylating agents.
Recently the characterization of a Alk⁻ mutant of E.coli has been described. This mutant exhibits increased sensitivity towards alkylating agents but not to UV or γ-ray irradiation. This mutant is though therefore to be defective in the specific repair of alkylation damage. We showed that the Alk⁻ mutant is much more sensitive to MMS than w.t. cells, whereas this difference does not excist for EMS. Furthermore the Alk⁻ mutant is hypermutable with MMS, whereas mutation induction with EMS occurs on the same level as in w.t. cells. The Alk⁻ mutant is probably defective in a pathway for repair of methylation damage. Using the LacI-system, the mutagenic spectrum for the Alk⁻ mutant after treatment with MMS was made and compared with the spectrum for w.t. cells.
Examination of these spectra revealed that mutagenesis on GC base pairs is not greatly affected by the alk mutation. However, it appears that 20% of the mutants induced in the cells of the Alk⁻ strain derive from substitutions in AT basepairs. In w.t. cells there is no significant mutation induction on AT basepairs after treatment with MMS. Therefore it seems likely that the repair pathway affected by the alk mutation operates on methylated AT basepairs.

b) Base analogs

Using the LacI-system, the mutagenic spectrum induced by N^4-hydroxycytidine was examined. It appeared that, in contrast to other known base-analogs, N^4-hydroxycytidine induces mainly if not exclusively AT → GC transitions. (7).

c) γ-irradiation

Examination of the mutagenic specificity induced by low (5kr) and high (30kr) dose γ-irradiation revealed that the spectrum changes with dose. Basepair substitution is the major type of mutagenesis after γ-irradiation and was shown to be RecA-dependent. (8).

d) Cis-$Pt(NH_3)_2Cl_2$

The mutagenic specificity of cis-$Pt(NH_3)_2Cl_2$ was examined.
Like several other cis-platinum compounds, cis-$Pt(NH_3)_2Cl_2$ exhibits antitumor activity, whereas the trans-isomer is ineffective. Platinum compounds exert their antitumor activity by inactivating the DNA-template and although the precise nature of the induced lesions is still unknown, it is thought that bifunctional binding to guanine

bases in the DNA is essential for their action. For this bifunctional mode of action several models have been proposed, which involve inter- or intra-strand crosslinks or interbase chelation on guanine bases in the DNA. It has been shown that a correlation exists between the induction of mutants in bacteria and the antitumor activity of plantinum compounds. Therefore examination of the nature of the mutants induced by cis-$Pt(NH_3)_2Cl_2$ might reveal the site of action of this antitumor compound.

Our results obtained sofar show that the specific action of cis-$Pt(NH_3)_2Cl_2$, as compared to the trans-isomer, is only clearly seen in repair deficient UvrB or RecA cells. Therefore the sensitivity of certain types of tumor cells towards cis-$Pt(NH_3)_2Dl_2$ might due to a repair deficiency in these cells.

Furthermore, we showed that cis-$Pt(NH_3)_2Cl_2$ appears to induce only basepair subsitution mutants in repair proficient cells. Probably the lesions which lead to this type of mutants are lethal in repair deficient cells. Since, at 6 % survival, 13 % of the induced mutants appear to be amber of ochre mutants basepair substitution mutagenesis forms a substantial part of the total mutagenesis induced by cis-$Pt(NH_3)_2Cl_2$. Examination of the nature of the induced basepair substitution mutants, using the LacI system, revealed that 70 % of these mutants arise from GC → AT or GC → TA substitutions at sites where the substituted guanine was part of a GAG or GCG sequence in the DNA. These results strongly suggest that basepair substitution mutants result from intrastrand crosslinks on two guanine bases separated by a third base. Since this type of mutants are not induced in UvrB or RecA cells, the repair of the proposed intrastrand crosslinks, leading to basepair substitutions, is probably dependent on both excision repair and a RecA mediated process.

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Title of Project Nr. 3 : The integration mechanism of bacteriophage Mu.

Head of Project and Scientific Staff : Dr. P. van de Putte, Dr. M. Giphart-Gassler, Drs. N. Goosen, Drs. T. Goosen, Drs. E. van Leerdam.

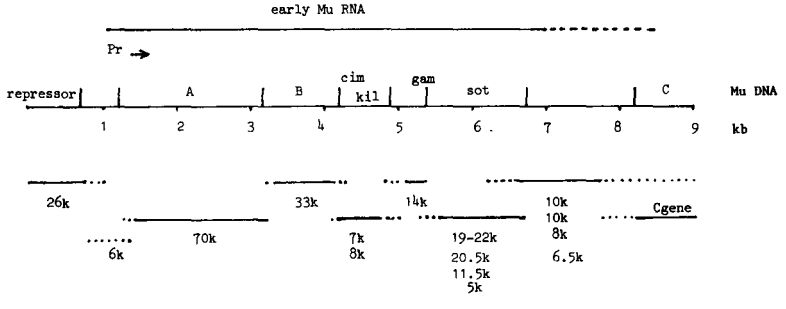
The mutator phage Mu causes polar mutations by random insertion into the genome of its host Escherichia coli by an illegitimate recombination process. In contrast to other phages this integration step is a prerequisite for Mu development and new rounds of integrations occur during Mu replication. The genetic rearrangements, such as deletions and inversions, found after thermoinduction of a Mu prophage show striking similarities with those found with other transposable elements (IS or Tn elements and retrovirusses). Genetic rearrangements are an important source of spontaneous mutations and, in some systems, probably also of radiation induced mutations. As transposition with Mu is a part of its effective replication machinery, Mu is used as a model system to study transposition and genetic rearrangements. Besides the recombination system for Mu integration, Mu has a second, although site specific, recombination system which can invert a part of its genome, the so-called G region. We have aimed during this 5-year contract period to study:

1. The Mu integration and replication process and the functions and structural sites involved.
2. The regulation of the Mu transposition process.
3. The functional role of the G region.

1a. Organization of early Mu genes and their expression

The genes essential for Mu integration and replication are located in the early region of the Mu genome. We have found by transcription studies in vivo and in vitro that this early region is transcribed as a single polycistronic messenger of approximately 6 kb long. Expression of late genes, involved in head and tail morphogenesis, is controlled by the product of gene C which we have shown to contain its own promotor (16). We have determined in the past that the two early genes A and B are essential for Mu integration and replication. Of these two, A is the Mu transposase and B the Mu replicase. By isolation of Mu mutants containing polar IS1 insertion we discovered four new non- or semi-essential early functions located beyond B. These are: cim, a function which stimulates repressor synthesis of Mu; kil a function involved in killing of host cells in the absence of the right end (β end) of Mu; gam, which codes for an anti ATP nuclease and sot, involved in the stimulation of Mu transfection (11-13). At present it is thought that one or more of these functions play a role in the regulation of the Mu transposition process.
To reach our intended goals concerning the study of early genes and their regulation it has been essential to obtain recombinant plasmids containing at

least the Mu genes A and B and their regulatory elements.
The plasmids pGP1 and pGP2 constructed in our laboratory have been of great value for further studies. These plasmids contain 5100 bp of the immunity end of the Mu genome linked to pMB9 and pBR322 (3,4,18). Due to the presence of a thermoinducible repressor activity the Mu genes A and B can be expressed at 42° C. Also the kil gene is expressed on these plasmids at 42° C. This property enabled us to isolate various kil⁻ plasmid mutants, insertion as well as deletion mutants (3,5). Moreover also host mutants were isolated in which the kil function is blocked (3). This hek (host expression of kil) mutation has been mapped between Dna G and arg G on the host chromosome. pGP1 subclones and mutants of pGP1 have also contributed to the physical mapping of early genes and their regulatory structures and have been used for the identification of gene products in E.coli minicells (see figure) (5,6).



The early Mu promotor-operator region (P_R O_R) has been mapped around the HindIII site, whereas the repressor promotor is located 900-1000 bp from the immunity end (5,16). We have determined that, in contrast to the remainder of the Mu genome the repressor is transcribed on the l strand (18), as found also for repressors of Tn elements.

1b. Integration and replication.

During the past 5 year period the problem of integration and replication has been studied in many laboratories. One of the main points resulting from these studies is that Mu replication involves a transposition process similar to that of other transposable elements. Experiments performed in our laboratory have contributed to a large extent to the models on transposition presented in 1979.

We have determined that Mu replication is unidirectional starting at or near the repressor end (8,21). We determined also that Mu replication stops at the β end and does not run into the adjacent DNA (8). As replication and reintegration are strongly coupled processes it has been impossible

to isolate replication intermediates. Also heterogeneous circles, containing E.coli DNA and Mu DNA, turned out not to be intermediates of Mu replication. Their origin is independent of the E.coli and Mu recombination systems (recA and gin) and should be explained by the transposition event itself.

The presence of the left Mu end and the expression of genes A and B are not sufficient for Mu replication. Neither defective prophages lacking the β end nor pGP1 containing cells do show any Mu specific replication. Plasmids derived from pGP1 containing also the β end (9) do, however, replicate after induction but only when the β end is correctly orientated towards the Mu c end. The Mu specific replication of such plasmids appears to be low compared with Mu phage replication, which could be due to the absence of one or more early functions.

Besides replication, pLP117 and its derivatives appear useful plasmids to study Mu integration. After induction at 42° C pLP117 disappears, presumably by integration in the host DNA. The same effect of induction has been observed in E.coli Dna B mutants, which suggests that integration and not replication is the first step in the transposition process.

2. Regulation of Mu transposition.

As products involved in transposition presumably interfere with the integrity of the host cell DNA, the transposition process should be strictly controlled. By studying the regulation of the expression of Mu early genes we might get better insight in how transposition in general is regulated and how transposase levels remain low.

Transcription studies have revealed that the total amount of early Mu RNA synthesis remains at a constant low level, independent of the number of Mu copies present (16). Also cells containing pGP1 show after induction this low transcription level despite the presence of 30 plasmid copies per cell (4). Thus, Mu contains besides the repressor an early gene with repressor activity. We could determine that this cro-like gene, ner, located between the repressor and gene A, negatively regulates transcription of early genes and negatively controls repressor synthesis. The Mu repressor synthesis is controlled positively by cim and is regulated by the repressor itself (autogenous regulation) (6). To study the regulation in detail various recombinant plasmids have been constructed in which various early genes were fused to the promotor of the kanamycin gene (P_{km}). Expression of Mu phage is blocked completely in cells containing P_{km} ner or P_{kn} ner$^+$A$^+$ plasmids. The pseudo-repressor activity of ner can be overcome only when both A and B products are provided. Plasmids containing P_{km} A, which leads to a constitutive expression of the Mu transposase, also interfere with the replication of Mu. The level of the Mu transposase is of extreme importance in transposition.

The availability of the plasmids described above and also of host mutants which fail to support growth of Mu enabled us to isolate specificly Mu regulation mutants. Some of them (pip mutants) show enhanced levels of early transcription (3,16). Preliminary sequence data indicate that these mutations are located in the early Mu promotor. Other mutants (ner-independent) have presumably mutations in binding sites for Mu repressors.

The study of Mu integration will be highly facilitated when we have purified the Mu transposase A. However, despite the fact that most of the regulation of early mRNA takes place at the level of transcription, the amount of A gene product, as appears on SDS gels, is very low compared to B gene product. Also no increased levels of A could be found when the A gene is fused to the km promotor. Our results therefore suggest that A is regulated also at the level of translation.

3. The function of the invertible G region.

The 3000 bp long G region of Mu is situated approximately 1700 bp from the right end of Mu and is flanked by short IR-sequences. Only Mu phage with the G region in the (+) orientation infects E.coli K12. We have determined that Citrobacter freundii, E.coli C and Shighella sonnei are also Mu sensitive and the infectious phage contains Mu DNA with the 'G' region in the (-) orientation. The invertible 'G' region therefore determines host specificity (14). We have substantial evidence that two sets of genes are located in 'G', one being expressed in the (+) the other in the (-) orientation. The products of these genes (at least three of them) could be identified in minicells with amber mutants of G^+ and G^- Mu phages. These and other observations have resulted in the presentation of two models for G organization. We are currently investigating the correctness of the models and the role of the gene involved in the inversion process. Gene regulation by the inversion of structural genes represents a novel regulation mechanism which may be operative in other organisms as well.

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Head of Research Team: Prof.Dr. F.H. Sobels

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Title of Project No. 1 : The genetic response of a D. melanogaster mutant strain (ebony) which is sensitive to UV and which has a low photoreactivating (PR) ability

Project Leader and Scientists: Prof.Dr. K. Sankaranarayanan and W. Ferro

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In 1978, Yegorova, Levin and Kozlova (Mutation Res. 49, 213-218) reported a study in which a mutant ebony and a wild-type (Canton-S) strain and their hybrids were compared with respect to UV sensitivity and PR ability. The eggs were UV-irradiated at the zygote stage (15 ± 2.5 min after oviposition) or at the blastoderm stage (180 ± 2.5 min after oviposition) and survival was measured with and without photoreactivation. From the results the authors concluded that: (i) the ebony strain was more sensitive to UV and also had a low PR-ability relative to Canton-S; (ii) the UV-sensitivity and PR-ability of the F_1 zygotes were different depending upon the nature of the cross: for instance the zygotes from e ♂♂ x CS ♀♀ were similar to CS while those from CS ♂♂ x e ♀♀ were similar to e, i.e., there was evidence for a maternal effect; (iii) the genes governing UV sensitivity and PR ability were probably located in the autosomes and (iv) the effects observed were not due to pleiotropic action of the e gene.

These two strains of flies were kindly provided by Dr. Levin. The aim of our work has been: (i) to investigate whether the greater UV-sensitivity and lower PR-ability of the ebony strain (with respect to killing) still persist in later developmental stages (i.e., first and second instar larvae) and to study the molecular biology of UV sensitivity in embryonic cells in vitro; (ii) to explore whether the ebony strain is also more sensitive to X-ray-induced larval killing and to X-ray mutagenesis; (iii) to check whether maternal effects of the kind noted by the Russian workers are also obtained for genetic end-points and (iv) to localize the genes involved.

UV-induced killing of first and second instar larvae and PR abilities

First and second instar larvae of the two strains (ebony and Canton-S) were irradiated with short-wave UV followed by exposure to PR light. After the completion of the treatment, the larvae were transferred to food vials

and allowed to complete their development. The ratio of the numbers of flies to those of larvae irradiated provided a measure of UV sensitivity. The results show that the ebony larvae are indeed more UV sensitive; however, with respect to PR ability, no firm conclusion can be made, as yet, in view of the variations between the replicate experiments.

X-ray-induced killing of first and second instar larvae

In experiments with X-irradiation, similar in design to those with UV, it was found that the ebony larvae are also more sensitive to the killing effects of X-rays.

X-ray mutagenesis

Irradiated (1000-3000 R) males of each of these strains were mated to females of each of these strains (in dominant lethal studies) or to Muller-5 females (in sex-linked lethal studies) or to Oster females (in sex-linked lethal and translocation studies) and sampling was restricted to mature spermatozoa. The results of dominant lethal studies show that mature spermatozoa from ebony males are more sensitive than those from Canton-S males and furthermore, the maternal effects of the kind noted by the Russian workers were also observed; the frequency of dominant lethality is highest when irradiated ebony males are mated to ebony females and lowest with the Canton S system; the reciprocal crosses fall in between.

In studies involving irradiation of stage 7 oocytes of e and CS females, (mated to Berlin-K males), the frequencies of dominant lethals in the ebony strain were consistently higher than in the other at all the exposure levels (1000, 2000 and 3000 R) tested.

The data on the induction of sex-linked recessive lethals demonstrate that mature spermatozoa of ebony males are more sensitive (1000, 2000 and 3000 R; several replicate experiments each involving between 600-1000 tested chromosomes per group per experiment). When irradiated Muller-5 males are mated to ebony or CS females and the induction of sex-linked lethals studied, it is found that these frequencies in general, are higher when ebony females are used.

Other results thus far obtained show that: (i) there is no sensitivity difference between the two strains with respect to the induction of autosomal translocations in mature sperm, the induction of X-chromosome losses in stage 7 and stage 14 oocytes and the induction of X-ray-induced ring-X chromosome losses. (In the last mentioned experiments, irradiated ring-X males were mated to ebony or Canton-S females.)

Gene localization

Strains containing chromosome 2 of the ebony strain and chromosome 3 of the Canton-S strain or chromosome 2 of Canton-S and chromosome 3 of the ebony strain were constructed and along with the parental strains tested for sensitivity to UV-induced killing of larvae and PR-ability. The limited data collected show that the strains containing chromosome 3 of ebony have a higher sensitivity to killing, i.e., similar to the parental ebony strain, which suggests that UV sensitivity may be due to a gene or genes located on chromosome 3; PR-ability, however, seems to be controlled by a gene or genes on chromosome 2, but more data would be needed to establish this.

Title of Project No. 2: Is there a proportionality between spontaneous and radiation-induced mutation rates?

Project Leader and Scientists: Dr. P.T. Shukla, Prof.Dr. K. Sankaranarayanan, Prof.Dr. F.H. Sobels and Dr. A. Schalet

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One of the methods currently employed in quantifying genetic hazards to humans from exposure to ionizing radiation involves the use of the so-called "doubling dose method". The doubling dose is the amount of radiation required to double the rate at which genetic events under consideration originate spontaneously and is estimated by dividing the spontaneous rate by the average rate of induction per rad of radiation. One of the assumptions implicit in the use of the doubling dose method for hazard evaluation is that there is a proportionality between spontaneous and induction rates; if this is not true, then it will be incorrect to apply the doubling dose across species boundaries (as it is done in estimating hazards to humans from mouse data). One way to test the validity of the assumption of proportionality between spontaneous and induction rates is to examine the average induction rates of mutations in related species or strains of organisms that differ from one another in their spontaneous rates. Another method is to examine, within a species, whether or not such a proportionality between spontaneous and induction rates is obtained on a locus-by-locus basis for a number of gene loci. The present work has employed the latter approach in two ways.

In one study, the X-ray-induction of recessive visible specific locus mutations at 14 X-chromosome loci was investigated using the "Maxy" technique. The X-ray exposure was 3,000 R to 5-day-old males and the sampling of germ cells was restricted to mature spermatozoa. Presumptive mutant females recovered in the F_1 generation were tested for transmission, allelism, fertility and viability in males. The different loci were ranked according to the number of mutants recovered and the rank order so established was compared with a rank order that can be established for the spontaneous mutation data at the same loci collected by one of us (A. Schalet, Ph.D. Thesis, Indiana University, 1960).

Considering first mutation frequencies and rates that can be estimated for the present data, a total of 128 mutations (115 completes and 13 mosaics) recovered among 38,898 female progeny were found to be transmitted.

On the basis of the above frequency, the average mutation rate can be estimated as 7.8×10^{-8}/locus/R; for mutations that were viable and fertile in males, the rate is 3.0×10^{-8}/locus/R. The frequency of mutations at the different loci encompassed a wide range; while no mutations were recovered at the raspberry and carnation loci, at others, the numbers ranged from 1 at echinus to 31 at garnet; in addition, the proportion of mutations that was male-viable was also different, depending on the locus.

Schalet's extensive data on spontaneous mutations at 13 (of the 14 loci) employed in the present study) loci permit an estimate of the spontaneous rate which is 6.1×10^{-6}/locus (a total of 39 mutations in 490,000 progeny); for mutations that were viable and fertile in males, the rate is 3.0×10^{-6}/locus. The mutability of the different loci varied over a nine-fold range.

When the different loci are ranked according to their relative mutability (for spontaneous and induced mutations), it is found that, in general, loci that mutate spontaneously relatively more frequently are also those at which more mutations have been recovered in the radiation study; likewise, those that are less mutable spontaneously are also those that mutate less after irradiation. Since these data are limited, it is concluded that the above finding is not inconsistent with the assumption of proportionality between spontaneous and induction rates of mutations. On the basis of the above results, a doubling dose of about 100 R can be calculated for the X-ray induction of specific locus mutations in Drosophila spermatozoa. These results have since been published (Shukla, Sankaranarayanan and Sobels, Mutation Res. 61, 229-248, 1979).

Another test of the validity of the assuption of proportionality between spontaneous and radiation-induced mutation rates was attempted by a comparison of spontaneous and induced X-linked recessive lethals. This was possible because of the availability of allelism data, (from Lefevre, U.S.A.), for X-ray-induced mutations, which consists of some 600 lethals distributed among approximately 250-300 loci in X-chromosome regions for which available duplications permitted allelism tests. During the period of this report we did not accumulate as many spontaneous lethals as we had expected to, so that the 155 spontaneous lethals from more than 95,000 tests yielded only 69 lethals tested for allelism in the "covered" regions studied by Lefevre. (23 additional lethals in "covered" regions not represented in the X-ray data were also subjected to allelism tests.) Among the 92 "covered" lethals, with the exception of a single locus, there were at most only 2 mutations per locus in 12 cases. The exceptional locus,

lethal J1 near the tip of the X-chromosome, which yielded 5 mutations including 3 that appear to involve small deficiencies, also mutates relatively more frequently with X-rays. Accordingly, the limited data from lethals agree with the results of the study with visible loci in being consistent with the assumption of proportionality between spontaneous and X-ray-induced mutation rates. The information obtained from the spontaneous lethals has been useful in providing a basis for comparison with the "MR" induced lethals (see project 7b) and useful in confirming the equivalence of average spontaneous rates for visible and lethal loci.

Title of Project No. 3: Radiation induced changes in meiotic and post-meiotic germ cell stages

Project Leader and Scientists: Dr. B. Leigh, Dr. R.H. Maddern, Drs. A.M.A.J. Veerkamp-van Baarle

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The basic theme of this project has been to investigate mechanisms underlying radiation induced chromosomal changes. 1) Oocytes were irradiated to study mechanisms of induced autosomal non-disjunction. 2) In another study oocytes were irradiated to induce compound autosomes and provide more information about selective processes that operate during the recovery. 3) The formation and recovery of rearrangements from irradiated spermatozoa has been considered in detail and the information gained has been used in other projects.

The aim of the non-disjunction study was to determine whether the mechanisms of induced sex-chromosome non-disjunction, proposed by Parker, can be used to explain the induction of autosomal non-disjunction. In the first part of the report period, earlier work was rounded off and this was reported in two publications (Leigh, 1979). An extension of the work required the construction of stocks with complex chromosome constitutions. This was necessary to permit an analysis of the products resulting from the induction of autosomal quasibivalents, i.e., the structures assumed to cause non-disjunction. In practice it was found that the stocks could not be put together. At critical points in the mating schemes lethality or sterility prevented completion. This part of the project has not been abandoned, but cannot be continued until a workable genetic scheme can be found.

Compound autosomes usually segregate at random during meiosis in the Drosophila male. During one of the earlier studies, on induced non-disjunction in oocytes, no exceptional progeny of the expected type were recovered when the treated females were mated to one specific type of male. This type of male carried the compound chromosome described as C(2R)RM,cn. Testing confirmed that these males produced regular gametes only. As a working hypothesis it was proposed that this compound contained an unusually long proximal duplication of 2L, which included one or more meiotic pairing sites. A series of new C(2L)RM chromosomes were induced by irradiating stage 7 oocytes and mating the treated females to males carrying C(2R)RM,cn. 20 of these chromosomes were tested with C(2R)RM,px, and 3 were inviable with this chromosome. These three chromosomes were used for the next stage of the experiment. Stage-7 oocytes of ORK females were irradiated and these females then mated to C(2L)RM, lethal; C(2R)RM,cn 1^{+} males. The total yield

was 154 C(2L) chromosomes and 12 new C(2R) chromosomes. Presumably both types of C(2) are induced at equal rates and the inequality is a secondary effect dependent on compatibility with the C(2) received from the unirradiated male. These data can be interpreted as providing support for the hypothesis that the induction of compound autosomes requires a break on either side of the centromere. Each new chromosome thus has a duplication/deficiency for proximal regions. These data have been published as an abstract (Leigh and Veerkamp-van Baarle, 1976).

Studies, on the fagments recovered in sex-chromosome loss experiments, were completed and the data published (Maddern and Leigh, 1976). A development of the model, proposed to explain the formation and recovery of compound autosomes, has been extended to account for the induction of centric fragments capped by a duplication of paternal chromosome material (Leigh, 1978). According to this model, radiation induces breaks in the chromosomes of the sperm nucleus. Repair occurs in the male pronucleus during part of the pronucleus S-phase. Some broken chromosome ends are joined together before replication and others after replication. This accounts for the production of both chromosome and chromatid rearrangements. An alternative hypothesis has been suggested and tested (see project No. 6). Publication of this work has focussed attention on the fact that the process of fertilization in Drosophila is not a unique process but most parts of the process are found in many organisms including mammals. At Oak Ridge (USA) it has been shown that irradiation of mouse spermatozoa is also followed by the formation of at least a few chromatid rearrangements.

Title of Project No.4.1: The effects of butylated hydroxytoluene on radiation-induced genetic damage in Drosophila germ cell stages

Project Leader and Scientists: Prof.Dr. K. Sankaranarayanan

Butylated hydroxytoluene (BHT) is a commonly used food-additive. With respect to its effects on radiation-induced genetic damage in Drosophila, conflicting reports have appeared in the literature. Kamra (Int. J. Rad. Biol. 23, 295-297, 1973) and Prasad and Kamra (Int. J. Rad. Biol. 25, 67-72, 1974) reported that injection of BHT into Drosophila males led to a sensitization of spermatozoa to the gamma-ray-induction of sex-linked recessive lethals, X-chromosome losses and autosomal translocations. Guzmán et al. (DIS 50, 140-141, 1973) reported that BHT had a protective effect in mature spermatozoa, i.e., the frequencies of sex-linked recessive lethals were lower in the "BHT+gamma-rays" group than in the "gamma-rays alone" group. Ives and Demick (DIS 51, 133, 1974) obtained clear indications that BHT acted as a radioprotector when Drosophila males were raised on a medium containing BHT, irradiated and then tested for the induction of translocations. The work reported below was initiated to resolve the contradiction between these different findings.

In the first set of experiments, wild-type Berlin-K flies were reared in a medium containing 0.05% BHT (in 0.5% DMSO) or in 0.5% DMSO alone and then tested for the X-ray-induction of dominant lethals (from treated mature spermatozoa, stage 7 and stage 14 oocytes) and sex-linked recessive lethals (from treated male germ cell stages). In the second set of experiments, female flies that were lethal-free for their second chromosomes were reared in the above two kinds of media and tested for the X-ray-induction of recessive lethals in their second chromosomes. In the third set, flies of a suitably marked strain were raised in the above media and the males were used to study the loss of their sex-chromosomes following X-irradiation.

The results demonstrate that: (i) the frequencies of X-ray-induced dominant lethals in mature spermatozoa and stage 7 oocytes are lower in the BHT-raised flies; such an effect is not observed in stage 14 oocytes; (ii) in sex-linked lethal and autosomal translocation experiments, BHT has a protective effect in germ cell stages sampled in brood C (corresponding to early spermatids); (iii) the frequencies of chromosome 2 recessive lethals were similar in both groups (stage 7 irradiation) and (iv) there were no significant differences in the frequencies of sex-chromosome losses in mature spermatozoa of males raised in the two media.

Title of Project No.4.2: The role of mutator genes on radiation-induced mutability in female germ cells of Drosophila

Project Leader and Scientists: Prof.Dr. K. Sankaranarayanan

The genesis of this work can be traced back to the publications of Green (Mutation Res. 10, 353-363, 1970), Green and Lefevre (Mutation Res. 16, 59-64, 1972) and of Gold and Green (Genetics 80, s35, 1975). The main findings, relevant for our investigation were the following: (i) the chromosome 3 mutator gene (symbol u) described by Green 1970 caused the recessive X-linked mutant genes y^2 and f^{3N} revert to wild type at a very high rate; the effect of the mutator was restricted to females; (ii) the mutator gene also increased the frequency of sex-linked lethals in females, but not in males; about half of the mutator-induced recessive lethals occurred in clusters, suggesting a premeiotic origin, and a sizeable proportion of these lethals were associated with cytologically detected chromosomal deficiencies; (iii) the X-ray-induced (100 R) frequency of sex-linked recessive lethals in u/u females was higher than in control (+/+) females; most of the lethals recovered in the former type of females occurred as clusters. The investigation reported below was aimed at verifying the findings with respect to the effects of radiation and extending the observations to other criteria of genetic damage and to other germ cell stages in the females.

From the stock containing the mutator gene (u/u), supplied to us by Prof. Green, a "control" stock was derived in which the mutator-containing third chromosomes were replaced by normal chromosomes. In these two stocks we studied the X-ray induction of sex-linked recessive lethals, sex-chromosome losses and dominant lethals in stage 7 oocytes, but only the X-ray induction of sex-linked recessive lethals in oogonial stages. (For oogonial experiments, 24 hr-old female pupae rather than young flies were irradiated.)

The results showed that: (i) the frequencies of induced chromosome losses were uniformly higher in the u/u stock than in the +/+ controls (5 exposures ranging from 750 R to 3,750 R and a total count of over 100,000 flies); (ii) the frequencies of radiation-induced dominant lethals and of sex-linked recessive lethals in stage 7 oocytes were similar in the two stocks; (iii) after oogonial irradiation (2,000 R), the frequencies of sex-linked recessive lethals, the number of clusters and cluster size distribution were all similar in the two stocks. The last-mentioned observation is at variance with what has been reported by Gold and Green (1975); it is not clear to what extent this result is due to the different exposures used in the present studies as compared to those of Gold and Green.

Title of Project No. 4a: Molecular nature of the lesions which lead to X-ray and spontaneous null-enzyme mutations: a combined genetic and biochemical investigation

Project Leader and Scientists: Dr. C.S. Aaron

The original long-term purpose of this project, initiated late in 1977, was to exploit the favorable biochemical features of the Alcohol dehydrogenase (Adh) locus and the selective methods available for detecting forward and reverse mutations in order to investigate the X-ray-induced and spontaneous mutation process in Drosophila melanogaster at the molecular level. Although a firm basis for such a study was provided by the work summarized below, the project was terminated in 1979 following the departure of Dr. Aaron from our laboratory.

The selective methods of Sofer were used to screen more than 300,000 progeny of X-rayed spermatozoa (500, 1000 and 3000 R) and more than 400,000 progeny in un-irradiated controls to establish induced and spontaneous forward mutation frequencies for transmitted mutants. The total induced null-enzyme mutation frequency was 7.1×10^{-8} per rad per locus, and the exclusion of those null-enzyme mutations associated with deletions yielded an induced "gene" mutation frequency of 3×10^{-8} per rad per locus. These data for a "biochemical" locus show good agreement with the average of the mutation frequencies found for 14 "visible" loci (see project 2) where the comparable figures were 7.8×10^{-8} per rad per locus and 3.0×10^{-8} per rad per locus. The estimated doubling doses for "gene" mutations at the Adh locus and the 14 "visible" loci are 73 R and 100 R respectively. The foregoing portion of the Adh work has been published (Aaron, Mutation Res., 63, 127-137, 1979).

The presumed Adh gene mutations recovered in these experiments were crossed inter se and with previously known complementing null-enzyme alleles and the resulting compounds were subjected to biochemical tests for allele complementation, but no complementation was detected among the induced mutants. However, immunological tests of 11 mutants provided strong evidence that at least 6 of them do, in fact, produce a non-functional protein, and therefore represent X-ray-induced intragenic changes.

Title of Project No. 5a: How are the marked sensitivity changes from immature (stage 7) to mature (stage 14) oocytes brought about?

Project Leader and Scientists: Prof.Dr. K. Sankaranarayanan

Most of the radiation-genetic studies with female Drosophila have focused attention on two stages, namely stage 7 and stage 14. Stage 7 oocytes are in prophase I of meiosis while stage 14 oocytes are in metaphase I of meiosis. These two manifest striking differences in radiosensitivity. For instance, for the X-ray-induction of dominant lethals, stage 14 cells are about 10 times more sensitive than stage 7 cells; for the induction of sex-linked recessive lethals, the former stages are about three times as sensitive as stage 7. The present investigation was aimed at examining whether the change in sensitivity between stage 7 and 14 is abrupt or gradual.

To sample oocyte stages between 7 and 14, the age of the female at irradiation was rigidly controlled. It is known that in a newly-eclosed female the most advanced stage is stage 7; in females aged two or more days, stage 14 oocytes, (the stage in which the eggs are laid), are found. Virgin females were irradiated with either 3,000 R of 500 R at different ages ranging from about 4 hrs after eclosion to about 48 hrs and the amount of radiation-induced dominant lethality was assessed following standard procedures in one set of experiments. In another set, females which were lethal-free for their second chromosomes were irradiated with 500 R of X-rays and the frequencies of chromosome 2 lethals were assessed.

The results showed, that for the induction of dominant lethals, sensitivity remains at a nearly constant level for oocytes sampled from females of ages 4 hrs to about 18 hrs; from then on there is a rather steep rise in sensitivity, (an increase of about 3% dominant lethality for every 1 hr increase in age), for oocytes sampled from females up to about 36 hrs. Beyond this, the increase, if any, is more gradual and a plateau is reached by the time the female is 48 hrs old. There is no further change in sensi tivity in oocytes sampled from females that were 72 hrs old.

The results of the recessive lethal tests show a similar pattern of sensitivity changes, although, as would be expected, it is less striking than that observed for dominant lethals.

Title of Project No.5b: Further studies on the mechanism of repair of chromosome breaks in Drosophila sperm, the role of repair processes

Project Leader and Scientists: Dr. B. Leigh

The construction of appropriate stocks is often a problem with Drosophila. This was the case with a project designed to provide more information about the observations of Falk (1962) and Sobels (1969), that enhanced frequencies of induced paternal autosomal translocations are recovered when irradiated male-germ cells are sampled using females with attached X.Y chromosomes. Wild-type ORK Drosophila melanogaster were given an exposure of 3000 R X-radiation. Mature sperm were sampled by mating to X.Y/X.Y, X.Y/X, or X/X females that carried markers on the second and third chromosomes for the detection of induced autosomal translocations. Two pairs of maternal stocks were used and heterozygous X.Y/X females were obtained by making both reciprocal crosses. The problem of stock construction was to obtain suitable combinations of X.Y chromosomes that suppressed crossing over in the heterozygote and yet carried similar combinations of markers on the autosomes. The highest frequencies of induced translocations were obtained with X/X females. In one series these frequencies were higher than those obtained with either X.Y/X or X.Y/X.Y females. In the other series a uniform frequency of translocations was obtained with all types of female, except for one of the two types of heterozygous female, which gave lower frequencies. This experiment has provided data which show that the addition of Y-chromosomes to the maternal genome does not have a specific effect on the recovery of induced paternal autosome translocations. Maternal Y-chromosomes increased the proportions of fertile F_1 males, this effect being consistent in direction but varying in degree. The data obtained in these experiments have been published (Leigh, 1979).

Title of Project No. 5c: The role of repair processes in the induction of mutations and chromosomal aberrations by X-irradiation

Project Leader and Scientists: Prof.Dr. F.H. Sobels, Prof.Dr. K. Sankaranarayanan, Dr. B. Leigh, Dr. J. Eeken, Drs. W. Ferro, Dr. H. Frei

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This project was initiated in 1979 because we expected that the newly available mutants and combinations of mutants deficient for biochemically well characterized repair processes would prove useful for a re-examination of our long-standing research interest in the study of factors (e.g. post-irradiation variations in O_2 concentration; caffeine or actinomycin effects) influencing the repair (in spermatids, spermatozoa or at the time of fertilization) of radiation-induced lesions which lead to mutations and chromosomal aberrations.

The bulk of our initial experiments involved the irradiation (X-rays or neutrons) in air, O_2 or N_2 of spermatids or spermatozoa in standard type ♂♂ which were subsequently mated to one of four kinds of ♀♀: 1) repair proficient; 2) excision-repair deficient mei-9, and two different types of post-replication-repair mutants, 3) mei-41, 4) mus-101. Three different types of genetic end-points were scored: 1) ring-X chromosome loss, which for the most part results from chromosome breakage related phenomena; 2) translocations, which are produced by chromosome breakage and rejoining, and 3) recessive X-linked lethals, which consist of point mutations and mutations associated with chromosome breakage and rejoining.

Table 1

	Lethals	Translocations	Ring-X Loss
mei-9	↑	-	↑
mei-41	-	-	-
mus-101	-	↓	↑

The first results for mature sperm irradiated in air are summarized in Table 1, which shows the magnitude of the X-ray-induced genetic damage relative to repair proficient controls, (↑ = larger; ↓ = smaller; = no difference), for each combination of repair deficiency ♀ used and genetic end-point scored. Thus, the biochemical characterizations which differentiated the 3 repair-deficient mutants is matched by a genetical characterization which discriminates among the 3 repair deficient mutants. The ability of the mei-9 and mus-101 mutant bearing ♀♀ to effect the magnitude of the damage indicates that the two repair processes for which they are deficient operate in repair proficient ♀♀ at the time of fertilization.

The first results from a series of experiments in which mature sperm from adult ♂♂ irradiated in air, N_2 or O_2 was sampled for recessive lethals after matings to repair proficient ♀♀ or mei-9 ♀♀ also yielded significant information. As compared to repair proficient ♀♀, more lethals were obtained from mei-9 ♀♀ after irradiation in air or N_2, but similar lethal frequencies were obtained after irradiation in O_2. These data suggest that there is a qualitative difference in the damage induced in air or N_2 vs. the damage induced in O_2, and are in accord with the interpretation of Sobels (Mutation Res., 2, 168-191, 1965) that lesions induced in O_2 are of a different kind. Furthermore, a limited amount of data was obtained from the irradiation of spermatozoa in N_2 followed by post-treatment in N_2 or O_2 and subsequent matings to repair proficient ♀♀ or mei-9 ♀♀. Although the data are insufficient to demonstrate the previously established contrasting effects of different post-treatments on the frequency of X-linked recessive lethals (Sobels, 1964, 1965), we can conclude, at least, that the defective repair process in mei-9 ♀♀ does not lead to an enhancement of the post-treatment effect.

The initial results from experiments to determine the manner in which mei-9 ♀♀ and mus-101 ♀♀ deal with X-ray damage as compared to neutron-induced damage in spermatozoa indicate that the neutron damage is being handled differently by these mutants. Especially noteworthy are the observations that mus-101 strongly reduces both lethals and translocations induced by neutrons, whereas, as shown in Table 1, mus-101 reduces translocations but is ineffective in modifying the frequency of lethals induced by X-rays.

Title of Project No. 6a: Exposure-fractionation effects for X-ray-induced dominant lethals in immature (stage 7) oocytes of Drosophila: a re-analysis

Project Leader and Scientists: Prof.Dr. K. Sankaranarayanan

Two of the main findings that have emerged from radiation studies with immature (stage 7; prophase I) oocytes of Drosophila are: (i) for acute X-ray exposures, the data on egg-survival give a satisfactory fit to a classical multi-target dose-response relationship defined by the equation:

$$\frac{S_D}{S_o} = 1 - (1 - e^{-KD})^N$$

where S_D = survival at dose D, S_o = survival in controls, K = target sensitivity and N = the number of targets to be hit (usually found to be not significantly different from 2) and (ii) fractionation of the X-ray dose into 2 or more fractions (with intervals of between 10 and 20 min) leads to an increase in egg survival relative to that after single acute exposures (Parker, Biol. Cont. Univ. Texas Publ. 5914, 113-127, 1959; Sankaranarayanan, Mutation Res. 25, 39-51, 1974; Traut and Schmidt, Int. J. Rad. Biol. 13, 405-415, 1968). Traut and Schmidt found that when X-ray exposures ranging from 1,000 R to 6,000 R were delivered singly or in two equal fractions (separated by 1 hr intervals) to stage 7 oocytes, the reduction in dominant lethal frequencies ("repair") was exposure-dependent because it decreased with increasing exposures. In addition, a comparison of the effectiveness of a single 6,000 R exposure with the same exposure delivered in either 2 or 6 fractions showed that, with 6 fractions, the amount of repair was higher.

Our investigation was designed to extend the observations of Traut and Schmidt on fractionation effects for X-ray-induced dominant lethals in stage 7 oocytes and more specifically to demonstrate that (i) the observed dose-dependence of repair recorded in their study is implicit in the kinetics of induction of dominant lethality in stage 7 oocytes, i.e., the dose-dependence is another way of describing a curvilinear dose response and (ii) the amount of observable reduction in dominant lethality (relative to unfractionated exposures) is predictable for any given fractionation regime from data on unfractionated exposures.

Young (0-4 hr old) Drosophila females were X-irradiated with single or fractionated exposures over a range up to 6,000 R and the induction of dominant lethality in stage 7 oocytes was studied. The results showed that:

(i) the frequencies of dominant lethals are higher after acute than after fractionated exposures; (ii) at any given exposure level, the higher the number of fractions, the lower the frequency of dominant lethals; (iii) the reduction in dominant lethality relative to single exposures increases with increasing number of fractions and (iv) this relative reduction in dominant lethality approaches a maximum value when the amount of X-irradiation approaches zero (i.e., when the egg-survival after single X-ray exposure approaches 100%); the maxima however, are different for the different fractionation regimes, being higher with increasing number of fractions.

Using statistical formulations it has been possible to show that the expected relative reduction in dominant lethality after fractionation is predictable from appropriate dominant lethal data on single exposures. These data have been published in full (Sankaranarayanan and Volkers, Mutation Res. 69, 249-262, 1980).

Title of Project No.6b: Investigation of the qualitative effect of neutrons

Project Leader and Scientists: Prof.Dr. F.H. Sobels and Dr. B. Leigh (with Dr. A.S. Robinson (ITAL), Dr. J.J. Broerse (TNO), Dr. A. Schalet, Dr. H. Frei, Drs. A.M.A.J. Veerkamp-van Baarle, and G.J. van Steenbrugge)

Comparative studies on the genetic effects of X-rays and neutrons were initiated in 1977. The practical aim was to find out whether neutrons might be more suitable for the induction of translocations to be used for animal and plant breeding. It is known that neutrons have a higher relative biological effectiveness for the induction of chromosome rearrangements. Because of the different induction kinetics, this advantage is greatest at low dose levels. The question asked was whether the rearrangements induced by neutrons have higher rates of associated recessive lethality than those induced by X-rays. This study was carried out in co-operation with Dr. A.S. Robinson using neutrons generated by the reactor of ITAL (Wageningen). Drosophila was selected as a test organism because of the easily available genetic schemes. The first part of the study was to establish a dose-frequency response for the induction of II-III autosomal translocations by the reactor neutrons.

The data gave the expected linear relationship with a remarkable consistency between two sets of radiations. For the comparison between neutrons and X-rays, males were constructed carrying lethal-free second and third chromosomes. The original experimental protocol consisted of inducing translocations and then testing translocated and non-translocated autosomes for the rates of lethality. This was found to be impracticable and only the induced translocations were tested for viability and fertility. 116 neutron induced and 152 X-ray induced translocations were tested as homozygotes and no significant difference was found in the ratio, lethal : fertile : sterile. The lethality could not be split into translocation - break-linked and translocation - independent components. With this qualification, it was concluded that similar rates of lethality are associated with the translocated chromosomes. This study has now been written up and will soon be submitted for publication.

The second comparative study on the genetic effects of neutrons and X-rays, was initiated from a theoretical question; whether chromosome and chromatid rearrangements are derived from double (DSB) and single (SSB) DNA strand-breaks respectively. This question came from the observation that X-irradiation of Drosophila spermatozoa induces both types of rearrangement. Recent cytophotometric studies have proven that sperm nuclei contain unreplicated chromosomes. Maddern and Leigh (1976) argued that, in the male pronucleus, repair of radiation damage and replication of the chromosomes are at least in part synchronous processes. An alternative explanation can be constructed on the basic assumption that the two types of rearrangement are derived from DSB's and SSB's. This can be tested by comparing the effects of types of radiation that are known to induce different DSB : SSB ratios. Neutrons and X-rays are two such types of radiation. High linear energy transfer (LET) neutrons induce relatively high rates of DSB's and low rates of SSB's. The genetic system used for this study was derived from the system used by Maddern and Leigh. Males carrying a doubly marked Y-chromosome were exposed to radiation and the progeny scored for losses and duplications of the Y-markers. At dose levels that yielded similar frequencies of exceptional progeny, the spectra of exceptional types were identical. This result has been interpreted as an indication that the chromosome and chromatid rearrangements were not derived directly from DSB's and SSB's, respectively.

These two studies were each designed to answer a specific question that formed part of the question about qualitative differences between the biological effects of neutrons and X-rays. The failure to demonstrate a difference in these two studies was considered a puzzle. It is known that there are qualitative differences between the primary effects of these two types of radiation. Nevertheless, taking into account other genetic studies reported in the literature, it appears very difficult to demonstrate any qualitative effects at the genetic level. The most positive report is contained in a book chapter by Muller (1954), but this does not contain any data. One of the simplest and most logical continuations of the comparative study was to repeat the experiment cited by Muller. This has now been started with an analysis of neutron induced mutations at the yellow, white, and forked loci. Preliminary data are interesting, but not yet sufficient to base any conclusions on.

Another approach is also being used. This is based on the knowledge that there are qualitative differences in the primary damage and indications from other studies that there are differences in the repairability of this damage. Using Drosophila sperm it is possible to treat a uniform population of nuclei and then by mating males to different kinds of females to expose this damage to variously modified repair mechanisms. Another project being carried out at this laboratory is concerned with the effects of repair deficient mutants. The question now being asked is whether modification of the oocyte repair system by these mutants causes a differential response to X-ray and neutron induced damage in spermatozoa. Data have been obtained with two mutants $\underline{\text{mei-9}}^{a}$ and $\underline{\text{mus-101}}^{D1}$. The genetic end points being studied are recessive sex-linked lethals and autosomal translocations. Qualitative differences have been found, but further work will be necessary to confirm these effects and to provide a basis for interpretation.

This series of comparative studies, on the genetic effects of neutrons and X-rays, originated with two specific questions. New approaches are beginning to provide information about differential effects of these two types of radiation. This project has achieved the aim of providing answers to the original questions and has provided a basis for planning new studies.

Title of Project No.7a: The effects of several X-ray qualities on the induction of genetic damage in the spermatocytes of Drosophila. The effect of oxygen tension on the induction of sex-chromosome losses in Drosophila males

Project Leader and Scientists: Drs. A. v.d. Wielen (with Prof.Dr. F.H. Sobels and Dr. B. Leigh)

Many "facts" become established in radiation genetics, indeed in science in general, when several investigators have obtained confirmatory data. The aim of this project was to investigate reports of data that did not conform to established expectation. One of the earliest observations of Muller was that over a wide range there is no effect of quality of X-rays on the induction of genetic damage. In 1971, Haendle reported an effect of X-ray quality on the induction of somatic recombination. One of the aims of this project was to find out whether this observation could be confirmed and extended to the induction of meiotic exchange, which is genetically more relevant.

The experimental protocol, designed for testing the effect of radiation quality on the rates of induced meiotic recombination, required the construction of stocks carrying suitable markers on the sex chromosomes and one of the autosomes. In practice a lot of time and energy was spent trying unsuccessfully to obtain these stocks. It was decided to use only markers on an autosome, the second chromosome. The 55 kV and 100 kV X-rays used were similar to the qualities of radiation used by Haendle. The data obtained did not provide any indication of a quality effect. Analysis did reveal that the distribution of crossovers varied according to the age of the time of radiation. The simplest way to sample irradiated spermatocytes is to expose young pupae, in which spermatocytes are the most advanced germ cell stage in the testis, and then sample the first sperm from the adult males. For technical reasons, there were unavoidable 2-5 hour variations in the age of the pupae at the time of exposure. In the youngest pupae there was a deficiency of induced centric exchanges. One experiment was considered insufficient to establish this interesting observation. Carefully timed pupae were irradiated at 2, 4, or 6 hours. The data showed a significantly different pattern in 2-hour old pupae. This finding indicates the complications that can arise when a rapidly developing system is irradiated.

In one experiment the induced recombinant chromosomes were tested for homozygous viability. Exchanges in the centric region, that is between black (b) and cinnabar (cn), had a higher frequency of homozygous viability than distal exchanges. This difference, however, was not significant.

This part of the project has been reported in Mutation Research, 59 (1979) 189-193. The most important observation is that there is no indication of a radiation-quality effect.

Baker (1957) reported that there is no oxygen enhanced effect for the induction of sex-chromosome loss when the sperm of R(1)1 (ring X-chromosome) and In(1)EN (rod X-chromosome) males are irradiated. Leigh has confirmed this observation with R(1)2 (ring X-chromosome), at the same time he showed that there was an oxygen enhanced effect for the induction of recessive sex-linked lethals and autosomal translocations in the same germ cell populations.

A common property, of the ring-X chromosomes and In(1)EN, is the large blocks of heterochromation on either side of the centromere. To find out whether this distribution is the critical factor, males carrying a variety of differently rearranged X-chromosomes were irradiated in oxygen or nitrogen and the progeny scored for sex chromosome loss. For 8 chromosomes the oxygen enhancement ratio was between 2 and 3. For In(1)EN a value of 1.7 was obtained and for R(1)2 a value of 1.2. This study confirmed that there is only a minimal oxygen enhancement effect on induced ring-X loss. The corresponding value for an opened ring (In(1)EN) was lower than that for other rod chromosomes, but the difference was not significant.

Title of Project No.7b: Analysis of mutations by a frequently occurring mutator gene

Project Leader and Scientists: Prof.Dr. F.H. Sobels, Dr. J. Eeken, Dr. A.P. Schalet

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A detailed analysis of the spontaneous mutation process is of considerable importance for understanding the origin of mutations and for being able to reliably relate induced mutations to spontaneous ones, as for example is being done by the doubling dose method. Recent investigations have shown that factors which greatly enhance the spontaneous mutation frequency, in an extremely specific way, are very common in nature. These factors are observed in bacteria, yeast, higher plants and Drosophila. In all natural populations of Drosophila melanogaster about half of the individuals appear to carry such a mutator gene (mutation-recombination factor "MR"). This MR factor causes visible mutations specifically at only a limited number of loci (including singed, raspberry, yellow and carmine) at a rate which is 500-1000 times higher than the ordinary spontaneous fre-

quency. The induction of X-chromosome linked recessive lethal mutations occurs with a frequency which is 4-5 times higher than the ordinary spontaneous frequency. The high locus-specificity for the induction of visible mutations by MR and the apparent instability of these mutations led to the hypothesis that MR causes breaks at specific sites, where, subsequently, insertion sequences become integrated.

The investigation during the past two years has been centred around the following questions.

I. Does there exist a locus-specificity for the induction of lethal mutations by MR and is the frequency distribution of these lethals comparable to the distribution as shown by ordinary spontaneous and radiation-induced lethals?

To analyse the distribution of MR-induced lethals, MR was introduced into males with the genetic constitution of y mei^{9a} mei^{41D5} (a double DNA-repair deficient mutant), y w In49 f or Berlin K (both repair proficient), and the induced X-chromosome linked recessive lethals were recovered. 7423 chromosomes of the double DNA-repair deficient, MR males were tested and yielded 106 X-chromosome linked recessive lethals (frequency 1.43%). 12563 chromosomes of the repair proficient, MR males were tested and 151 lethals were recovered (frequency 1.35%). Nearly 150 of these 257 lethals are covered by duplications of the X-chromosome and could be further localized using duplication/deficiency mapping and tested for allelism with ordinary spontaneous or radiation-induced lethals. Despite the still relative small number of MR-induced lethals collected versus the number of lethal genes on the X-chromosome (a maximum of 800) a number of loci could be identified showing more than 1 MR-induced lethal where only 1 or no lethals were found in the sample of ordinary spontaneous lethals (Table I). These results suggest that there exists a locus-specificity for the induction of lethals by MR and that the frequency distribution is different from the distributions shown by ordinary spontaneous and radiation-induced lethals.

II. Does the MR factor influence the frequency of radiation-induced lethals?

To investigate the interaction of MR with radiation-induced lesions, the induction of X-chromosome linked recessive lethals was measured in irradiated and non-irradiated MR males, and an irradiated (2000 R) non-MR control group. The frequencies of lethals observed are: non-treated MR males 0.9% (55/6167), treated MR males 7.3% (343/4693) and treated non-MR males 5.5% (279/5062). The irradiation of MR males yielded significantly more lethals than the sum of the lethals produced by MR alone and those obtained from the irradiated non-MR males.

III. What is the effect of DNA-repair deficient mutations on the action of MR?

To examine whether excision and incorporation of insertion sequences by MR is possibly associated with enzymatic pathways involved in DNA repair, the following experiments were carried out. MR was introduced into males deficient for excision (mei^{9a}) or post-replication repair (mei^{41D5}) or into males carrying both repair-deficient mutations. MR activity was recorded by the induction of visible mutations at the sn (singed) and ras (raspberry) loci. The ordinary spontaneous mutation frequency for sn is $0.2 \cdot 10^{-5}$ (1/490000) and $0.4 \cdot 10^{-5}$ (2/490000) for ras (Schalet, Proc. Xth Int. Cong. Genet. (Montreal) Vol. 2, 252, 1958). When MR is introduced into repair-proficient males the frequency of sn mutations is $51 \cdot 10^{-5}$ (16/30795) and of ras $19 \cdot 10^{-5}$ (6/30795). In males deficient for both excision and post-replication repair (mei^{9a} mei^{41D5}) MR mutator activity is significantly enhanced; in two independent experiments the frequency of sn is $304 \cdot\cdot 10^{-5}$ (93/30542) and $239 \cdot 10^{-5}$ (73/30554) and of ras is $170 \cdot 10^{-5}$ (52/30542) and $134 \cdot 10^{-5}$ (41/30554). By contrast neither mei^{9a} or mei^{41D5} alone resulted in such a dramatic change of the MR-induced mutation frequencies; with mei^{9a}, $123 \cdot 10^{-5}$ (50/40583) sn and $54 \cdot 10^{-5}$ (21/40583) ras mutations were recorded, and in combination with mei^{41D5}, $111 \cdot 10^{-5}$ (30/27127) sn and $52 \cdot 10^{-5}$ (14/27127) ras mutations were observed. It would thus appear that the mutator activity of MR is significantly increased in the presence of both repair-deficient mutations as compared to the frequencies of MR-induced mutations in the presence of each repair-deficient mutation alone. In order to see whether, perhaps, the combination of mei^{9a} and mei^{41D5} was responsible for the observed enhancement, sn and ras mutation frequencies were determined in double mutant males without MR; no sn and only 2 ras mutations were found among 119671 tested chromosomes. The mechanism of mutation induction of MR seems a rather specialized form of mutagenesis as expressed in its extraordinary locus-specificity and the very nature of the mutations themselves. However, we have clearly shown that modified DNA-repair pathways influence the mutator activity of MR and that vice versa the presence of MR affects the rate of repair of X-ray induced damage. This indicates a similarity in the enzymatic pathways of ordinary DNA-repair and MR directed mutagenesis.

Table I. Comparison of the distributions of MR induced lethals (sample size 257) and ordinary spontaneous lethals (sample size 150)

Region	MR-induced alleles	spontaneous alleles
1A6	0,1 [1)]	5
1F4	3	1
2E1-2F5	3	0 or 1
3C7	> 5	2
6C11-7A2	4	0 or 1
7B3-7C1	2	0 or 1
7C4-7C9	2	0, 1 or 2
10C2-10E2	2	0
11A2-11A7	3	0, 1 or 2
19E6-19E8	2	0
rest	0 or 1	0 or 1

[1)] The sample size of MR-induced lethals including locus 1A6 is 173

Title of Project No. 8: Development and improvement of mutational assay systems in mammalian cells.

Project Leader and Scientists: Drs. A.G.A.C. Knaap, Drs. A.J. de Kok, Dr. J.W.I.M. Simons, Dr.Ir. A.A. van Zeeland

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For a reliable assessment of the radiation induced mutation frequencies in mammalian cells it is necessary to determine dose-response relationships for mutations induced in a variety of different loci. For the validity of the extrapolation of the data obtained with somatic cells to damage induced in germ cells it is furthermore necessary to compare the mutation induction in a number of cells from different origins. Therefore the research during the contract period was focused on the development of mutational assay systems, which would allow the determination of induced mutation frequencies at different loci and in different species. The markers under study were HGPRT (hypoxanthine-guanine-phosphoribosyl-transferase)-deficiency, TK (thymidine kinase)-deficiency and APRT (adenine-phosphoribosyl-transferase)-deficiency. These markers were investigated in three cell lines: human diploid skin fibroblasts (HSF), L5178Y mouse lymphoma cells and V-79 Chinese hamster cells.

Preceding this contract period our laboratory had already determined the mutation induction per rad at the HGPRT-locus in V-79 Chinese hamster cells (1.3×10^{-7}/rad) and in L5178Y mouse lymphoma cells (1.4×10^{-7}/rad). These determinations became possible via extensive studies on the time needed for the expression of induced mutants. As at that time the expression times found (6-8 days) appeared surprisingly long, experiments were carried out with L5178Y cells on the effect of drug concentration on the length of the expression time and it was determined how long after irradiation mutations became fixed. The results were in agreement with a mutational origin of the mutants. In HSF the expression of HGPRT-deficient mutants appeared shorter (3 days) and the mutation induction per rad amounts to 2.3×10^{-7}. This suggests that the human cells have a somewhat higher mutation induction than rodent cells. At the same time the mutation induction per rad for widely different cells from widely different species are remarkably similar.

The marker for HGPRT is X-linked and therefore per cell only one active gene copy is present. To study mutation induction for an autosomal recessive marker it is a prerequisite to obtain a cell line first with only one intact gene. A mouse lymphoma cell line was received, which was presumably heterozygous for TK. A mutational assay has been developed with these cells. The expression time for induced mutants proved to be 2 days whereas for HGPRT 7 days were needed. The mutation induction per rad was 2.1×10^{-7} for TK and 1.1×10^{-7} for HGPRT.

Experiments have further been carried out to develop mutational assay systems for APRT. A human cell line heterozygous for APRT-deficiency became available derived from skin biopsies of the parents of an APRT-deficient patient. The conditions for the mutational assay system have been sorted out. Data on X-ray induced mutant frequencies are not yet available. In V-79 Chinese hamster cells and L5178Y mouse lymphoma cells rare double mutants, completely deficient in APRT, have been isolated. Until now it appeared not possible to select revertants from these cells, which would have possessed one active APRT-gene and could have been used in subsequent mutation studies. Another approach is now being followed in which, by the use of moderate toxic concentrations of the selective agents, mutants have been isolated with reduced APRT levels. It will be investigated whether this reduction in APRT is due to the loss of one APRT-gene.

Another objective has been the development of a mutational assay system using cells directly from the animal so that comparisons can be made between cells which have been treated in vivo within the organism and cells which have been treated when cultured in vitro. First mouse bone marrow cells have been used. Although the conditions for culturing these cells have been sorted out and the conditions for selection of mutants were probably such that mutants would have grown into visible clones no HGPRT-deficient mutants have been observed. An explanation could be that the HGPRT-gene is essential for the formation of bone-marrow colonies.

Secondly cells have been used, which had been taken from 12-day old embryos from the golden hamster. These cells proved to be useful and the conditions for the selection of mutants from these cells (e.g. cell culture methods, drug concentration during selection, cell density during selection, degree of metabolic corporation, expression time) have been sorted out.

Title of Project No. 9a: Correlation between chromosome aberrations, mutations and cell killing

Project Leader and Scientists: Dr. P.P.W. van Buul, Dr. A.T. Natarajan, Dr. J.W.I.M. Simons, Dr. A.D. Tates, Dr.Ir. A.A. van Zeeland

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For a quantitative comparison between the induction of mutations and chromosome aberrations in vitro, it is essential to synchronize the cultured cells with respect to the cell cycle. So far no method for the synchronization of cells has been sufficiently successful. Therefore the rate of mutation induction in V-79 Chinese hamster cells was compared with chromosome aberration frequencies in the same cells using different sampling times for the determination of chromosome aberration frequencies. The ratio between breaks per cell and mutations per cell was 25000 after 400 R, 450 after 2.5 J/m^2 and 1150 after 5 J/m^2. This suggests that the mechanisms underlying mutation and chromosome aberration induction are at least partly different and that simple correlations between chromosome breakage and mutation induction probably do not exist.

Experiments with fractionated doses of UV (2.5 J/m^2 + 12.5 J/m^2) separated by a 2 hour interval of UV suggested also that the response for mutation is different from that for the induction of chromosome aberrations.

Title of Project No. 9b: Studies on the discrimination between point mutations and deletions in mammalian cells

Project Leader and Scientists: Drs. A.G.A.C. Knaap, Dr. J.W.I.M. Simons

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Mutations at the HGPRT locus can be generated both by base changes and small deletions. Knowledge about the origin of the observed mutants will be important to obtain a better insight into the mutational process and is also of importance for the evaluation of genetic risk because small deletions are supposed to constitute a greater genetic risk than base changes. Mutants caused by base changes are in principle revertible, whereas mutant caused by small deletions will be non-revertible. Reverse mutation was shown to occur in a HGPRT-deficient mutant, which had been induced by the mismatching agent 6-hydroxy-amino purine. The conditions for the quantitative determination of reversion frequencies will be sorted out.

Another approach to the same problem involves the staining for G6PD. If large deletions are viable, the gene for G6PD, which is located on the X-chromosome close to HGPRT, might be deleted together with the gene for HGPRT. The presence of G6PD can be ascertained by a staining reaction in which nitroblue tetrazolium is reduced to formazan. This method has been applied to V-79 Chinese hamster cells. Reconstruction experiments in which G6PD-deficient clones were present among G6PD-proficient clones failed to produce a clear distinction between the two types of clones, although cultures of both types of cells can be distinguished easily. This latter observation will allow the test of a limited number of HGPRT-deficient clones for the presence of G6PD.

Title of Project No. 10: Studies on chromosomal non-disjunction
Project Leader and Scientists: Dr. A.D. Tates

After the development of a cytological technique for the detection of sex-chromosome non-disjunction and diploid spermatids in male germ cells of the field vole Microtus oeconomus (Tates et al., 1975) we set up a breeding colony and carried out a series of experiments using X-rays and a group of chemicals as potential non-disjunction inducing agents. The summary of results in this report only pertains to studies with X-rays. In the early series of experiments we demonstrated that X-rays (25, 50, 100 and 200 rad) significantly increased frequencies of non-disjunction and diploid spermatids (Tates et al., 1979; Tates, 1979; Tates, 1980). Data from these experiments suggested variations in sensitivity between different germ cell stages. Furthermore, it was found that more non-disjunction was induced in the early experiments and that some males were more sensitive than others to the induction of non-disjunction and diploid spermatids. At the time these experiments were carried out, the number of available animals in the newly established Microtus colony was quite small, especially in the initial experiments. Therefore, it was possible that the observed variabilities were at least partly attributable to the small sample size in the early experiments.

Recently, a much larger experiment has been carried out which involved 4 different doses of X-rays (25, 50, 100 and 200 rad), 6 different sampling times (1, 4, 6, 7, 9 and 12 days after irradiation), and 6 animals for each combination of dose and sampling time. In contrast to earlier findings, there was no induction of non-disjunction above control levels. A possible explanation for the differences in results between earlier and later experiments might be that genetic changes have taken place in the Microtus colony that was initiated with animals trapped in the wild, but which now has become highly inbred. In the same large experiment where non-disjunction induction could not be detected it was found that diploid spermatids were frequently induced. The dose-effect relationships for the different time intervals were linear, but the slopes were different, indicating stage-specific differences in sensitivity. The average doubling dose is of the order of 12 rad with a range of between 5-30 rad for the individual time intervals.

Recently, a new breeding colony has been started, but the animals for this colony come from another region of Scandinavia than the animals used for the old colony. Preliminary data from an experiment with the new colony seem to indicate that non-disjunction induction is negligible, and diploid spermatids are frequently induced. Thus, the prospects for studying radiation-induced non-disjunction in the new colony are not encouraging.

Title of Project No. 11: Structural chromosome aberrations in somatic and germ cells of mammals

Project Leader and Scientists: Dr. P.P.W. van Buul, Prof.Dr. A.T. Natarajan and Dr. A.D. Tates

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The potential of using the chromosomal radiosensitivity of mammalian somatic cells for assessment of genetic radiation risks in man has extensively been investigated. In the mouse the induction of reciprocal translocations in bone-marrow cells was compared with that in spermatogonia (as scored in the descending spermatocytes). Dose-response relationships (0 - 600 rad X-rays) were constructed for both cell types (van Buul, 1977a) and the effect of changes in the exposure-rate (0.0287 - 130 rad/min γ-rays) was studied (van Buul and Roos, 1977). The results obtained can be interpreted as indicating that dividing somatic cells react qualitatively in a similar way as mitotically dividing premeiotic male germ cells, when scored for the same type of chromosome aberrations (van Buul, 1976c, and van Buul, 1976d). Frequencies of X-ray-induced dicentric chromosomes and deletions were studied in peripheral blood lymphocytes of man, the rhesus monkey and different mouse chromosome mutants. The following mouse stocks were used (a) normal laboratory mice; (b) T70H translocation heterozygotes; (c) tertiary trisomic mice bearing an extra T(1:13)70H translocation chromosome but with a normal phenotype; (d) T(1:13)70H tertiary trisomic mice with a clearly aberrant phenotype (teeth trisomics) and (e) tobacco mice (Mus poschiavinus), with 26 chromosomes instead of the normal mouse chromosome number of 40. The mouse work (100, 150 and 200 rad X-rays) indicated no differences between normal mice, translocation heterozygotes and trisomic mice of normal phenotype, whereas the induction of dicentrics was higher in the 'teeth' trisomic mice and the deletion induction was higher in the tobacco mouse (van Buul et al., 1977). In further investigations we were unable to confirm the results obtained with the 'teeth' trisomic mice (van Buul and de Boer, 1977). The results with monkey and human blood lymphocytes show about equal radiosensitivities for these species when cells were analysed at their first division after stimulation only (van Buul, 1976b, and van Buul et al., 1980b). In addition the human data indicated that the mixing of first and second cell cycle cells at the usual fixation times

cannot explain the observed variation in the frequencies of chromosomal aberrations but that donor-to-donor variation is the predominant factor influencing yields of aberrations. The condition of a donor seems to be most important because repeats with the same donor also showed marked variability (van Buul and Natarajan, 1980). Comparison of aberration frequencies obtained in mouse and man showed that normal mice were as equally sensitive to the induction of dicentrics as man thus refuting the "arm number" hypothesis of Brewen et al., 1973 (van Buul, 1977b).

Translocation induction in stem-cell spermatogonia in most of the above mentioned mouse 'chromosomal mutants' revealed that only the translocation heterozygotes were clearly less sensitive whereas all the other strains had similar radiosensitivities (van Buul and de Boer, 1977). These results suggest that the sensitivity of a given mammalian species to the induction of dicentric chromosomes after in vitro irradiation of peripheral blood lymphocytes may not be indicative of the recovery of induced reciprocal translocations from its spermatogonia.

The induction of reciprocal translocations in stem-cell spermatogonia of the rhesus monkey (Macaca mulatta) was studied after single (50, 100, 200, 300 and 400 rad) or fractionated (200 + 200 rad with 24 h interval) X-ray exposures. The observed frequencies of aberrations were 0% at 0 rad, 0.46% at 50 rad, 0.86% at 100 rad, 0.99% at 200 rad, 0.68% at 300 rad and 0% at 400 rad, suggesting a humped dose-response relationship without a dose quadratic component of the yield in the pre-humped dose range (van Buul, 1976b and van Buul, 1980). These frequencies were lower than those reported for the other investigated primates, the marmoset and man. At present it is not clear whether the differences are attributable to true species differences or to differences in technical procedures. The recovery of translocations after dose-fractionation (0.71%) was higher than after exposure to the total single dose (0%), but much lower than expected on the basis of additivity of the two parts of the dose (1.98%). These data suggest a difference in split dose effects in the rhesus monkey compared to the mouse, the guinea pig and the golden hamster. Long term recovery experiments in the rhesus monkey, using doses of 300 or 850 rad X-ray and recovery times of from 4-13 years, demonstrate that even 7 years after an exposure to 300 rad a highly significant increase in the translocation frequency can be observed (up to 4.6% due to clonal proliferation), but that after 850 rad no translocations could be recovered.

For both the rhesus monkey and the normal laboratory mouse, the ratios between the frequencies of dicentric chromosomes in blood lymphocytes and the frequencies of reciprocal translocations in stem-cell spermatogonia were calculated at doses of 50, 100 and 200 rad X-rays (van Buul et al., 1980b). In the mouse this ratio was about 4 but in the rhesus monkey this ratio is greater than 10. These results, together with the earlier mentioned ones for the T70H translocation heterozygous mouse, strongly suggest that for mammals the chromosomal radiosensitivity of blood lymphocytes does not seem to have a predictive value for the frequency of recovered translocations from stem-cell spermatogonia. Hence, in our opinion only direct observations on induced chromosomal aberrations in germ cells of higher primates and man can play a decisive role in estimating human genetic radiation risks arising from chromosomal aberrations. Recently some experiments were initiated to investigate the effects of low doses (25 rad) and of low dose rates (0.2 R/min) on translocation induction in rhesus monkey spermatogonia. The induction of translocations in stem-cell spermatogonia of the marmoset (Callithrix jacchus) is also under investigation. 6 marmosets received single doses of 50, 100 and 200 rad X-rays and most of them showed reasonable recovery 1-2 years after irradiation. Some castrations have been made and the scoring of slides is in progress. From part of the rhesus monkeys and all marmosets castrated so far smears from epididimal spermatozoa have been made to analyse for sperm head abnormalities, but the scoring of these has not yet been started.

A program to analyse induced translocations in testis biopsies from human patients exposed to therapeutic X-ray doses is, due to its nature, progressing very slowly. So far a total of 132 cells from 4 different irradiated donors and 84 cells from 2 different controls have been analysed, but no translocation configurations have been detected.

Translocation induction in stem-cell spermatogonia of multi-generation irradiated mice (200 rad X-rays per generation for 69 generations) turned out to be the same as in the controls (van Buul, 1976a); therefore no sensitization or resistance could be demonstrated. Spermatogonia consist of heterogeneous cell populations with respect to cell killing and the induction of chromosomal aberrations. To get more insight into this complex

system fractionation experiments were performed in the mouse both for translocation induction in stem-cell spermatogonia analysed at the spermatocyte stage many cell generations after induction and for chromosomal aberration induction in mitotic dividing spermatogonia immediately after irradiation. Previous studies (van Buul and Leonard, 1974) have shown that a small conditioning dose of X-rays (100 R) sensitizes the spermatogonial population to the induction of translocations by a second one applied 24 h later. In follow up studies (van Buul and Leonard, 1979, and 1980) we demonstrated that sensitization does not occur when the first exposure is lower than 100 R and the threshold dose for sensitization must be between 75 and 100 R. Similar experiments with other strains of mice and other radiation circumstances indicate that these factors probably have an effect on the level of the threshold dose. Experiments on mitotically dividing spermatogonia revealed that with this approach no sensitization could be demonstrated (van Buul and Zwetsloot, 1980). Extensive fractionation studies with continuous sampling of cells (0-48 h) combined with cell proliferation analysis via *in vivo* BrdU chromosome labelling lead us to the conclusion that the sensitization effect as observed for translocations and mutations is characteristic for the stem-cell spermatogonia and does not occur in the more differentiated premeiotic male germ cells. With the aid of bleomycin we were able to show that in contrast to most chemical mutagens, so-called radiomimetic agents are able to induce reciprocal translocations in the genetically important stem cell spermatogonia (van Buul and Goudzwaard, 1980).

In germ cells, reciprocal translocations (symmetrical aberrations) are often used to measure genetic damage, whereas in somatic cells dicentric chromosomes (asymmetrical aberrations) are most frequently used for this purpose. Therefore the ratio between X-ray-induced symmetrical and asymmetrical chromatid exchanges was studied in Chinese hamster cells *in vitro* and *in vivo*. The results indicate that this ratio is independent of the stage of the cell cycle in which the aberrations are induced (van Buul et al., 1977, and 1978), but that addition of Neurospora endonuclease seems to favour the formation of asymmetrical exchanges.

In our rhesus monkey spermatogonial studies the induction of translocations by X-rays turned out to be correlated with the FSH blood level at the time of irradiation. Efforts to change the radiosensitivity of mouse spermatogonia by chronic treatment with FSH have been negative but *in vivo* experiments with pituitary deficient dwarf mice and preliminary *in vitro* experiments with Chinese hamster cells suggest a possible relation between hormones and the induction of chromosomal aberrations (van Buul et al., 1980a).

Title of Project No. 12: Molecular aspects of the origin of chromosomal aberrations with special reference to DNA repair.

Project Leader and Scientists: A.T. Natarajan, A.A. van Zeeland, P.P.W. van Buul

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Introduction

About 12 in 1000 human newborns have numerical or structural chromosomal aberrations and a large proportion of spontaneous abortions in humans are associated with chromosomal aberrations. In mammalian cells ionizing radiations are very efficient in inducing chromosomal aberrations, but very poor in inducing point mutations. Thus, genetic hazards due to ionizing radiation in humans are more likely to appear in the form of chromosomal aberrations rather than point mutations. An understanding of the basic mechanisms involved in the formation of chromosomal aberrations, especially following ionizing radiations, is very important and this project was started with the aim of elucidating these mechanisms. We have tried during this report period (1976-1980), to quantify DNA primary lesions, their repair and correlate with biological endpoints, such as chromosomal alterations including sister chromatid exchanges. In addition to ionizing radiations, ultraviolet rays of different wavelengths were also used in some experiments, in view of the specificity with which short wave UV induces pyrimidine dimers in DNA, and the accuracy with which the fate of pyrimidine dimers can be monitored.

In this project we have employed the following approaches, utilizing: 1) DNA repair deficient or mutagen sensitive human cell lines, mainly radiosensitive ataxia telangiectasia (AT); 2) mammalian cells made permeable for large molecules (endonucleases) following irradiation; 3) cell lines such as fibroblasts from chicken embryos or Xenopus laevis, which possess photoreactivating enzyme, capable of photoreactivating UV induced pyrimidine dimers; 4) cells which have been sensitized to ionizing radiations and UV by growing in medium containing 5-bromodeoxyuridine (5-BrdUrd) for one or two cell cycles prior to irradiation.

1) Studies with repair deficient cell lines

It is known that cells derived from patients suffering from the human recessive disease Ataxia telangiectasia are more sensitive to the biological effects of ionizing radiations when compared to normal cells. Biochemical investigations have not been able to detect any specific defect in DNA repair in these cells, except in some cell lines, there was a deficiency in repair of base damage induced by X- or γ-rays. However, cytologically AT cells respond with higher frequencies of chromosomal aberrations for a given dose of X-rays, when compared to normal human cells. In contrast to normal cells, which respond with chromosome type of aberrations (dicentrics, rings and chromosome fragments) when cells in G_0 or G_1 stage are irradiated, AT cells respond with both chromosome and chromatid types of aberrations (Natarajan and Meijers, 1979). Studies involving ionizing radiations, UV and alkylating agents, have shown that damages which require a DNA replication dependent repair ('S' dependent) lead to chromatid type of aberrations even after treatment in G_1 stage, whereas damage which can be repaired independent of 'S' stage lead to chromosome type of aberrations and chromatid type of aberrations following treatment in G_1 and G_2, respectively. On this basis, after irradiation of G_1 cells of AT we would expect increased frequencies of chromosomal aberration, especially of the chromatid type, if the aberrations were due to defective repair of X-ray-induced DNA base damage, a type of lesion which requires on 'S' dependent repair. We would not expect an increased frequency of aberrations following G_2 irradiation of AT cells. In fact, it was found that G_2 cells from AT were also more sensitive to aberration induction by X-rays. This indicates that probably a defect in repair of X-ray induced DNA strand breaks is responsible for the increased sensitivity of AT cells (Natarajan and Meijers, 1979). In this study it was also found that AT cells are extremely sensitive to induction of chromosomal aberrations induced by β-rays from incorporated tritiated thymidine (Natarajan and Meijers, 1979). Experiments with neutrons (0.5 MeV, with an RBE of 7 to 10) indicate that irradiation of G_2 cells induces about a 10-fold increase in the frequencies of chromosomal aberrations in AT cells when compared to normal human cells. This magnitude is similar to that obtained following X-irradiation (Natarajan et al., 1981). These results imply a defect in the repair of DNA double strand breaks in AT cells, as DNA double

strand breaks have been shown to be responsible for the production of X-ray induced chromosomal aberrations (Natarajan and Obe, 1979; Natarajan et al., 1980a).

If DNA strand breaks are not effectively repaired in AT cells, then more breaks should be available for the influence of agents which interfere with repair, such as caffeine, in AT cells compared to normal cells. Experiments to check this possibility were conducted with peripheral blood lymphocytes of AT patients and normal individuals in which G_0 cells were irradiated with X-rays and challenged at different times (representing G_1, S, and G_2) with 10^{-3} M caffeine (Natarajan et al., 1980c). These results demonstrated that the frequencies of X-ray induced chromatid and isochromatid breaks could be effectively increased in AT cells irrespective of the cell stage in which the post treatment with caffeine was done, and indicate lesions sensitive to caffeine persist for a long time in AT cells.

From the accumulated cytogenetic data we conclude that (a) compared to normal human cells, AT cells are more sensitive to all types of radiations tested so far, and (b) the defect in AT cells is probably a deficiency in repairing DNA strand breaks.

2) Studies with permeabilized cells to identify the primary DNA lesions involved in the production of chromosomal aberrations induced by ionizing radiations

Among X-ray induced DNA lesions, single strand breaks (SSB), double strand breaks (DSB) and base damage have been implicated in the formation of chromosome aberrations in eukaryotic cells. The facts that (a) the cells are extremely efficient in repairing single strand breaks, and (b) densely ionizing radiations are very efficient in producing chromosome aberrations (high RBE) have lead to the concept that DSB's are the important lesions leading to chromosome aberrations. There was no direct experimental evidence for this. To elucidate this problem, we have exployed mammalian cells which have been made permeable to large molecules following treatment with inactivated Sendai virus. Chinese hamster ovary cells (CHO) in G_2 stage have been irradiated with X-rays (50, 100 and 200 R) and immediately treated with inactivated Sendai virus and Neurospora endonuclease (E.C. 3.1.4), an enzyme that specifically cleaves single stranded regions in DNA. X-rays induce 10 to 20 SSBs to 1 DSB, and it is expected that during the repair of X-ray induced SSBs, small stretches of single strand regions (gaps) will become available and some of them will be converted into double strand breaks by

the Neurospora endonuclease (NE). The results obtained showed that the frequencies of X-ray induced aberrations (gaps, breaks, exchanges) increase 3 to 4-fold by the Neurospora endonuclease. This indicates that some of the single strand breaks are indeed converted into double strand breaks by this enzyme (Natarajan and Obe, 1978). When cells which have been irradiated with 0.5 MeV neutrons in G_2 are post-treated with NE, there was no increase in the frequencies of all classes of chromosomal aberrations. Thus, this enzyme cannot affect the yield of neutron induced chromosomal aberrations. This is not unexpected as densely ionizing radiations induce predominantly DNA double strand breaks.

Employing neutral sucrose gradient centrifugation we have also measured the induction of DNA double strand breaks induced by X-rays in CHO cells, with and without NE post-treatment under conditions in which the cytological experiments were conducted. The number of double strand breaks induced by X-rays increased by a factor of 2, following NE treatment (Natarajan et al., 1980a), an increase similar to the one obtained in cytological experiments.

Taken together these results can be interpreted to indicate that the major DNA lesion induced by ionizing radiations which leads to chromosomal aberrations is a DNA double strand break.

Using the above technique we have investigated the duration of the availability of single strand gaps for the action of NE, in G_2 cells following X-irradiation. The results showed that such gaps are available for the action of NE till prophase of mitosis; however the proportion of breaks vs. exchanges varied and depended on the time of treatment with NE. The prophase cells showed a dramatic increase in breaks, however there was no increase in the frequencies of exchange type of aberrations. This indicates that the breaks are spacially separated from each other due to condensation of chromosomes, and are unable to interact with each other to give rise to exchange aberrations (Natarajan et al., 1980c). NE treatment in G_2 did not influence the frequencies of aberrations in cells which were irradiated in early or late S phase, which indicates that no single strand gaps were available for the action of NE, and all breaks are efficiently repaired in S (Natarajan et al., 1980a).

When synchronized G_1 cells are X-irradiated and post-treated with NE, in addition to an increase in the frequencies of chromosome type of aberrations, we also observed a proportional increase in chromatid type of aberrations. This mixture of chromosome and chromatid type of aberrations obtained after G_1 irradiation is similar to that obtained in AT cells

following G_1 or G_0 irradiation (without NE post-treatment). This may indicate some single strand lesions not repairable in G_1 persists till S to be repaired or misrepaired during replication and give rise to chromatid type of aberrations (Natarajan et al., 1980a).

3) Role of pyrimidine dimers in UV-induced chromosomal aberrations and sister chromatid exchanges (SCEs)

The major DNA lesion responsible for most of the biological effects following short wave UV (254 nm) is probably a pyrimidine dimer. Direct evidence for this came from photoreactivation experiments in which it was shown that, when cells were exposed to visible light following UV irradiation, the biological effects induced by UV were reduced. While some data on the effects of photoreactivation (PR) exist for the induction of chromosomal aberrations by UV, there have been conflicting reports about (PR) in the literature on the induction of SCEs. UV-induced pyrimidine dimers can be quantified by treating the DNA from UV irradiated cells with UV endonuclease from T_4 to induce breaks - near the dimers, and the efficiency of this technique has been improved in our laboratory by employing freezing and thawing of the cells (van Zeeland et al., 1980a). Using this technique, we have quantified the number of pyrimidine dimers in CHO cells, following irradiation with UV of different wavelengths, namely 254 and 290. The doses were adjusted to get a similar number of dimers for both types of UV and the frequencies of SCEs were determined in the same experiment (Reynolds et al., 1979). The frequencies of SCEs increased linearly with the increase in the number of pyrimidine dimers induced, irrespective of the wavelength of the UV light employed, which indicates that pyrimidine dimers are most probable lesions responsible for the SCEs induced by UV. In order to obtain further evidence for this, we employed chicken embryonic fibroblasts, which are capable of photoreactivating UV-induced pyrimidine dimers (Natarajan et al., 1980c). The number of pyrimidine dimers was quantified before and after PR, and at the same time the frequencies of chromosomal aberrations and SCEs were determined. These results indicated that on photoreactivation there was a reduction in the frequencies of dimers, chromosomal aberrations and SCEs. Chicken embryonic fibroblasts are characterised by (a) small chromosomes, including a large number of microchromosomes, which are difficult to analyse cytologically; and (b) the frequencies of SCEs tend to saturate around 25/cell if the large chromosomes alone are scored. Accordingly, it is necessary to work at very low doses. In order to overcome these difficulties,

we employed cells from Xenopus laevis which also possess PR enzyme. In an extensive study using these cells we have demonstrated that PR reduces the frequencies of UV-induced pyrimidine dimers as well as all biological effects studied, namely cell killing, chromosomal aberrations, SCEs and point mutations to ouabain resistance (van Zeeland et al., 1980b). This is the first demonstration of the influence of PR on UV induced point mutations in higher eukaryotes. These result indicate that following UV irradiation all biological effects so far studied are probably induced by pyrimidine dimers; however, not every dimer gives rise to a biological effect since large number of dimers (about 20,000 per SCE) are needed for a single event. This would indicate that the location of the dimer in the DNA (linkers vs. nucleosomes) as well as its interference in DNA replication are important factors contributing to the biological effects observed.

4) Studies on the mechanisms of chromosomal aberration formation using cells with chromosomes containing 5-BU substituted DNA

By growing cells in a medium containing 5-BUdR for one or two cycles, or one cycle in 5-BUdR followed by another cycle in thymidine, one can obtain cells containing chromosomes in which the chromatids are of different constitutions, namely TT-TT (thymine-thymine), TB-TB (thymine-bromouracil), TB-BB, TB-TT. Incorporation of BU into the DNA of a chromatid alters the staining property, as well as increasing the sensitivity to chromosome-damage by UV and X-rays. The same X-ray dose produces about 3 times more breaks in a unifilarly BU substituted chromatid than an unsubstituted one. Long wave UV (350-390 nm) produces aberrations exclusively in a chromatid containing BU substituted DNA (Kihlman et al., 1978). On this basis, we developed a system in which we can induce mainly X-rays lesions or exclusively long wave UV lesions in one of the two sister chromatids of a chromosome, provided that one of these chromatids contains unifilarly 5-BU substituted DNA, while the other contain unsubstituted DNA. The sister chromatids of these chromosomes can be distinguished by differential staining (Kihlman et al., 1978, Natarajan et al., 1980d). In Vicia faba root tip cells and also in CHO cells, we found that the sensitivity to the production of aberrations by X-rays increased with an increase in the number of 5-BU substituted DNA strands, i.e. TT-TT $<$ TT-TB $<$ TB-TB $<$ TB-BB. Using this system we investigated whether a damaged chromatid may interact with an undamaged one to form an exchange by making use of the finding that in cells with chromosomes of TT-TB constitution, long wave UV produced no breaks in TT chromatids whereas

X-rays produced about 3 times as many breaks in TB as in TT chromatids. The results of both UV and X-ray experiments clearly indicated that two lesions are required to produce one exchange; this provided direct evidence in support of the conclusion derived indirectly long ago from the dose-effect relationship for radiation-induced exchanges (Kihlman et al., 1978).

Title of Project No. 13: Studies on the induction of pyrimidine dimers, cell killing and mutation induction by sunlight

Project Leader and Scientists: Drs. P.M. Burger, Dr. J.W.I.M. Simons and Dr.Ir. A.A. van Zeeland

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In principle it is possible to predict the mutagenicity of sunlight or of a solarium lamp when the spectral composition of the light source and the relative effectiveness of light of different wavelengths in inducing mutations is known. This was investigated by measuring the mutation induction and the induction of pyrimidine dimers by monochromatic light of 254, 297, 302, 313 and 334 nm. These data were used to construct an action spectrum for the induction of mutations and endonuclease sensitive sites. These action spectra were subsequently used to predict the mutagenicity of the Philips TL-12 solarium lamp. The data indicate that the number of endonuclease sensitive sites induced by the solarium lamp can be estimated from the action spectrum for endonuclease sensitive sites, but that the number of induced mutations is underestimated. An underestimation appears to be present also for the estimation of the mutation induction by sunlight. The data obtained so far suggest that the action spectrum for endonuclease sensitive sites can be used to predict the mutagenicity of a solarium lamp.

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Contractor: State University of Leiden
Department of Physiological Chemistry

Contractnumber: 193-76 BIO N

Projectleader: Prof.Dr. A.J. van der Eb

General subject: Studies in the mechanism of repair of radiation damage in mammalian cells

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Title of project 1: Studies in the mechanism of repair of radiation damage in mammalian cells

Projectleader and scientist: Prof.Dr.A.J. van der Eb, Dr. P.J. Abrahams

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We have investigated a number of genetic diseases in man which are characterized by defective repair mechanisms of DNA damage. The method used to study these syndromes consists of determining the extent of host-cell reactivation of SV40 virus or SV40 DNA treated with radiation or a DNA damaging agent, after infection of the mutant cells. (Abrahams and Van der Eb, Mutation Research 35, 13-22 (1976). The following types of cells were used:

1. Xeroderma pigmentosum (XP), 7 complementation groups. In all XP lines tested as well as in a XP variant line, reactivation of UV-irradiated SV40 was reduced to different extents.
2. Ataxia telangiectasia (AT), 3 cell strains were tested, using OsO_4-treated SV40 as the probe (OsO_4 causes damage in DNA resembling X-ray damage). All 3 cell strains showed a decreased capacity to reactivate OsO_4-treated SV40. This work will be extended by using bleomycin-treated SV40 DNA. An alternative possibility to use BK virus as a probe rather than SV40 virus will be investigated (BK virus can be assayed on human cells by direct plaque-titration, in contrast to SV40 which can only be assayed using an infectious-centre assay.
3. Cockayne's syndrome. Survival of UV-irradiated SV40 was tested in one cell line; the survival was found to be reduced in this line compared to that in normal cells.
4. In order to study cells from Fanconi's anemia (FA) patients which are defective in their ability to repair cross-links in DNA, conditions are being tested to introduce cross-links in SV40 DNA by exposure to psoralene + 360 nm UV light.

These studies form the basis for experiments in which genetic heterogeneity will be investigated by testing host-cell reactivation in heterokaryons of representative cell lines of a particular genetic syndrome (AT, FA or Cockayne's syndrome).

Contractor: State University of Leiden
Department of Physiological Chemistry

Contractnumber: 248-77-1 BIO N

Projectleader: Prof.Dr. A.J. van der Eb

Scientist: Dr.J.H. Lupker

General subject: Studies on the relationship between repair mechanisms of radiation damage in DNA and oncogenic transformation.

The purpose of this project was to search for inducible and error-prone repair mechanisms in mammalian cells, comparable to "SOS-repair" in E.coli, and to study the possible relationship, with induced reactivation and mutagenesis on one hand and the sensitivity to oncogenic transformation on the other hand.

Inducible reactivation of UV-irradiated SV40 in monkey cells.

The studies on the occurrence of inducible repair mechanisms of UV-damage were carried out with BSC-1 monkey cells, using SV40 virus as the probe. It was found that the survival of UV-irradiated SV40 is higher in cells that were treated with a low UV dose than in untreated cells. Similar results were obtained when SV40 DNA was used rather than intact virus, suggesting that reactivation acts on the viral DNA and is not a consequence of enhanced virus adsorption or uptake. Enhanced reactivation is maximal when infection is delayed for 2-3 days after irradiation of the cells. It was also observed that UV-treated cells produce more virus (i.e. allow more viral replication - rounds) than unirradiated cells. The time course of this process is similar to that found for enhanced virus reactivation which suggests that facilitated virus production and reactivation of damaged virus are manifestations of the same process.

UV-induced mutagenesis in SV40 after replication in monkey cells.

Studies were also carried out to determine whether irradiation of BSC-1 or CV-1 monkey cells with a low UV dose results in an increased mutagenic activity, as measured by the rate of reversion to the wild

type (wt) phenotype of a temperature sensitive (ts) SV40 mutant. The production of wt revertants in the progeny of unirradiated ts BC245 was followed by infecting cells at various times after infection. Mutation frequency reached a maximum value when infection was delayed for 3-4 days after irradiation of the host cells and declined gradually thereafter. Virus grown in unirradiated cells did not show such an alteration in the mutation frequency. The temporal increase of the mutation frequency of SV40 in UV-irradiated cells is due to a transient mutagenic activity rather than to an increased number of replication rounds in UV-irradiated cells. This suggests that signals are produced in UV-treated cells which are responsible for the observed generation of mutations in undamaged virus.

The generation of mutants following irradiation of the virus alone is greater when infection is delayed for some days after the cells reached confluency. When, in addition, the host cells were irradiated with UV,no further increase of the rate of mutation was observed.

Since similar time courses were obtained for reactivation of UV-damaged SV40, capacity of virus production and mutagenesis, it is suggestive that these phenomena are manifestations of the same general process, which possibly may be an inducible error-prone repair mechanism. Further studies are in progress to determine whether introduction of UV-irradiated non-viral DNA into monkey cells also results in an increased mutagenesis in the progeny of unirradiated SV40 virus.

Inducible and error-prone repair processes in human cells.

We have also investigated whether human cells contain inducible and error-prone repair mechanisms similar to those detected in monkey cells (see above). It was found that the survival of UV-irradiated SV40 DNA was higher in UV-pretreated human cells than in untreated cells. Enhanced reactivation is maximal when infection was delayed for 48 h post-irradiation of the cells, suggesting that the activity was induced by UV treatment. The kinetics of this process are very similar to those in monkey cells suggesting that the phenomena are manifestations of a similar mechanism. To investigate whether exposure of human cells to low UV doses also induced a mutagenic activity, the rate of reversion of the SV40 ts BC245 mutant to the wild type phenotype is presently being studied, both in UV-irradiated and unirradiated human fibroblasts.

Preliminary results suggest that such an activity may indeed be present in UV-irradiated fibroblasts.

Conditions are also being worked out to identify inducible and error-prone repair mechanisms in repair-deficient cells (XP, AT). If such activities are found we will investigate whether these pathways play a relatively more important role in repair-deficient cells than in normal cells.

Preliminary experiments have been carried out to assess the relationship between induced repair or mutagenesis on one hand, and oncogenic transformation on the other. A reproducible method to assay transforming activity in human cells has been worked out, in which SV40 DNA cloned in a bacterial plasmid was used as transforming agent.

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Contractant de la Commission: Université Libre de Bruxelles

N° du contrat: 156-76-1 BIO B

Chef des groupes de recherche: Jean Brachet

Thème général du contrat: Effects of irradiation on the stability and expression of genetic information.

Titre du projet N° 1: Effects of ionizing radiations on nucleic acids as studied by Electron Spin Resonance.

Chef du projet et collaborateurs scientifiques: A.J. Bertinchamps; S. Gregoli, M. Olast and R. Mathur - De Vré.

The eventual effects of radiation on living systems result from a complex chemistry induced on their constituent macromolecules virtually at the time of exposure. The least interference with the intricate pattern of these radiation--induced chemical reactions may have extreme consequences on the eventual response of the whole organism to radiation. Although a protection against the deleterious effects of ionizing radiations is theoretically possible at each stage of their cellular and somatic development, chemical protection at the level of primary chemical effects represents the earliest external intervention which may successfully be accomplished. Thus, the relevance to radiation protection of studies aiming at an elucidation of the molecular mechanisms governing the so-called "Primary Effects" and their possible control by means of suitable non toxic additives is self-evident and does not need to be further emphasized.

In the framework of the concerted research programme established by the members of the "European Group for the Study of the Primary Effects of Ionizing Radiations on Nucleic Acids", our planned contribution consisted of ESR studies of the free radical intermediates produced by γ -rays on DNA, their reaction pathways and their dependence upon several chemical and conformational factors. To attain this objective, the complexity of the system studied was progressively increased, from the constituent nucleotides through their random stacking complexes to the DNA molecule itself. The result obtained at each step of the research are briefly summarized below.

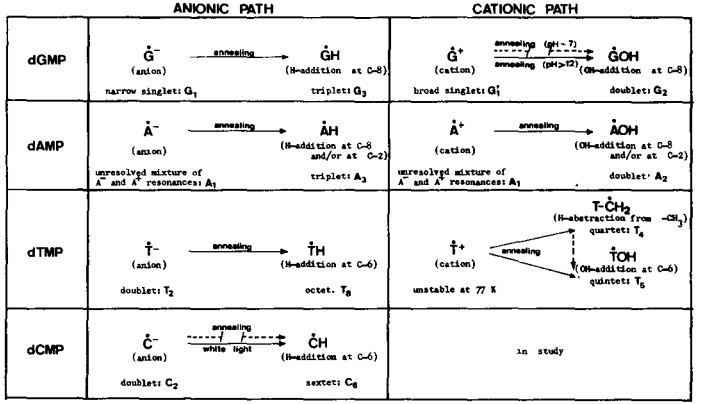
1) γ-radiolysis of mononucleotides in frozen aqueous solution.

dAMP and dGMP. The two purine nucleotides may be treated together owing to the great similarities in their radical structures and reaction pathways. Irradiation at 77 K produces in both cases radical anions ($\dot{A}^-$, $\dot{G}^-$) and radical cations ($\dot{A}^+$, $\dot{G}^+$). All these species are stable at 77 K and their ESR spectra have been resolved. With increase in temperature, ionic radicals progressively enter conversion reactions: $\dot{A}^-$ and $\dot{G}^-$ protonate at C8 to form the corresponding H-addition radicals (ȦH, ĠH); $\dot{A}^+$ undergoes OH-attachment to C8, (ȦOH), while $\dot{G}^+$ decays without apparently converting into a secondary radical. On the other hand, since at alkaline pH both $\dot{A}^+$ and $\dot{G}^+$ do transform into the corresponding OH-addition radicals (ȦOH, ĠOH), we conclude that this reaction pathway is not forbidden to radical $\dot{G}^+$, but that, at neutral pH, there is a preferential pathway, probably involving non radical species.

dTMP. Irradiation at 77 K produces anionic ($\dot{T}^-$) and cationic species ($\dot{T}^+$). Under heat treatment, $\dot{T}^-$ undergoes protonation at C6 to form the corresponding H-addition radical (ṪH). The conversion pathway of $\dot{T}^+$ is less clear. First of all, $\dot{T}^+$ is not stable at 77 K, such that conversion products should already be formed. In fact, an ESR pattern, probably associated with the radical of H-abstraction from the methyl group at C5, is detected at 77 K. This radical may be considered as the secondary radical from $\dot{T}^+$. However, with increase in temperature, another radical is progressively formed: this radical has unambiguously been identified as the radical of OH-addition to C6 (ṪOH), another very strong candidate to be the secondary radical from $\dot{T}^+$. Another possible explanation could imply a two-step conversion reaction of $\dot{T}^+$: deprotonation of the methyl group, $T(\dot{C}H_2)$, and conversion of this radical to the radical of OH-addition to C6 (ṪOH). The mechanism of the later reaction is, however, unclear.

dCMP. Irradiation at 77 K and further annealing produces composite ESR spectra which are not resolvable in terms of elementary components. The only unambiguous finding is that $\dot{C}^-$ radicals are formed and stabilized at 77 K. Their protonation at C6 is possible if the activation energy is furnished in the form of white light illumination. This pathway is in fact forbidden under thermal annealing which seems to favour a conversion reaction involving non-radical species.

All the results described above are summarized in the following Table.



The ionic character of these species refers to their π-electron systems (π-ions) and does not correspond necessarily to the net charge of the entire molecule.

2) γ-radiolysis of random costacking complexes of DNA nucleotides.

The data concerning the γ-radiolysis of single nucleotides in frozen aqueous solution has been used to elucidate the energy degradation pathways in more complex systems, such as the random costacking complexes obtained by freezing at 77 K aqueous solutions containing different nucleotides in different molar proportions. The overall conclusion was that the radiation chemistry of these heterogeneous systems cannot be considered to be simply the sum of the radiation chemistries of the constituent subunits. More specifically, it has been shown that:

(i) important charge migration phenomena take place in all the nucleotide complexes: positive charges migrate toward guanine, while negative charges migrate toward cytosine.

(ii) the above point is consistent with the assumption that electron affinity increases in the order G < A < T < C, with the converse true for electron donor properties.

(iii) as a consequence of charge redistribution, a large excess of cytosine anions and of guanine cations is formed. Secondary radicals and end-products are therefore selectively localized on the same constituents.

3) Radiolytic pathways in γ-irradiated DNA. Influence of chemical and conformational factors.

This point was the ultimate objective of our research programme outlined in 1976. No comprehensive knowledge was indeed available at that time: the results in the literature being highly conflicting and arising from analyses of data obtained under disparate and sometimes extreme experimental conditions. After a very systematic study of the different DNA building blocks of increasing complexity, our experimental approach thus culminated in a detailed investigation of the DNA molecule itself.

As for the previous investigations, the work on DNA was also heavily grounded on the application of a computer-assisted technique for the analysis of composite ESR spectra developed in our laboratory. This permitted us to elucidate the mechanisms by which free radicals are formed in the DNA macromolecule at yields depending on several chemical and conformational factors, so producing extreme variations in the resulting ESR spectra. Nevertheless, computer-assisted analysis of all of these spectra revealed the following common composition: a doublet from thymine anions ($\dot{T}^-$); a singlet from guanine cations ($\dot{G}^+$); an octet from 5-thymyl radicals (ṪH) and an asymmetric doublet from thymine located peroxyl radicals (TOȮ). This formal interpretation of the experimental spectra (see Fig. 1), permitted us to elucidate the following basic features for DNA radiolysis in frozen aqueous solution:

- Neutral DNA solutions frozen at 77 K are phase-separated systems. Radiation damage to DNA does not result from the indirect effects of radiation.

- Primary radicals on DNA are ions of both signs, randomly produced on the constituent bases.
- Charge migration occurs via the stacked bases. Thymine is the eventual sink of the long-range electron migration. Guanine is the eventual sink of the short-range positive hole migration. Migration phenomena may be partially or totally hindered by various interfering factors.
- With increase in temperature, ionic radicals enter conversion reactions: $\dot{G}^+$ decays without reacting with the surrounding water molecules, whereas $\dot{T}^-$ does react to form the 5-thymyl radical.
- In the presence of oxygen, peroxyl radicals $T\dot{O}O$ are formed rather than 5--thymyl radicals.
- No sugar-located free radicals have been detected in the course of the present investigation.

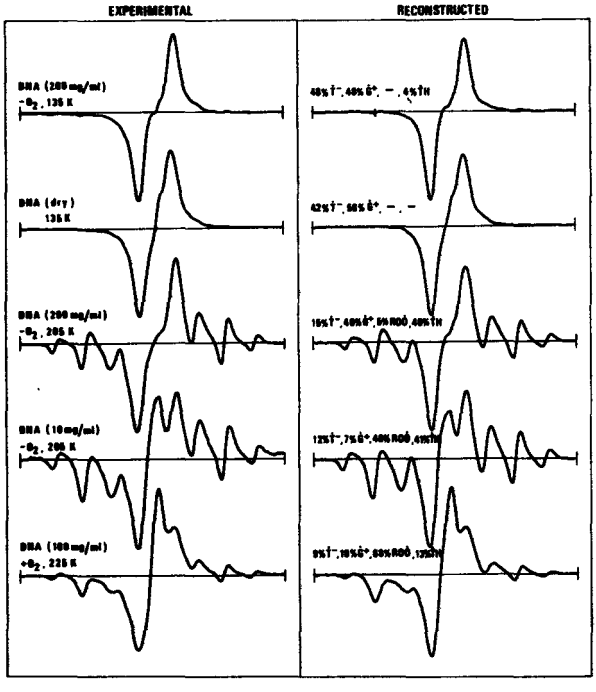


Figure 1.

Reconstruction of typical DNA spectra by means of linear combinations of the pure elementary patterns associated with thymine anions ($\dot{T}^-$), guanine cations ($\dot{G}^+$), 5-thymyl radicals ($\dot{T}H$) and peroxyl radicals ($R\dot{O}O$) respectively. Before reconstruction work, the four input patterns were normalized with respect to a common double-integral value. In the reconstructed spectra, the relative weights of the spectral components also give the radical yields of the corresponding radicals.

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Titre du projet n°2 : Genetics and biochemistry of DNA repair, mutagenesis and recombination in procaryotes and eucaryotes

Chefs du projet et collaborateurs scientifiques : M. ERRERA et M. RADMAN, P. CAILLET-FAUQUET, J. CORNELIS, G. MAENHAUT-MICHEL, A. BROTCORNE-LANNOYE, J. ROMMELAERE, P. JEGGO, A. KINSELLA, S. OSBORN, A. MOGG, C. DINSART, J.M. VOS, LU ZU YU, SU ZAO ZHONG

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1) Microorganisms

During the last four years the research of our group was aimed at elucidating the phenomenology of the repair of radiation damage to DNA, induced and spontaneous mutagenesis, and recombination in procaryotes and eucaryotes. We have further investigated the genetic controls of the inducible error-prone DNA repair (SOS repair) which is mainly responsible for the mutagenic effect of radiations and of a great majority of chemical mutagens (1,5,6,7,12). The results of our attempts to identify inducing signals for the mutagenic functions in the irradiated cells allowed us to conclude that cellular "alarmons" (Ames) such as cyclic nucleotides (AMP cyclic or ppGpp) do not play a critical role in the induction of SOS mutagenesis (Caillet-Fauquet, Defais-Villani, Brotcorne-Lannoye, to be published). Using lysogenic cells permeabilized by toluene treatment, we have developped a method to detect the expression of one of the SOS functions (i.e. the inactivation of the λ prophage repressor). We were not able to observe any inducing effect on the level of λ repressor by metabolites of DNA replication such as dXMP or dXTP.

Genetic and biochemical studies of mutagenesis led us to postulate the existence of an SOS inducible factor which suppresses a fidelity function in the replicative complex. This fidelity function was originally supposed to be the proof-reading 3' to 5' exonuclease (3). We have developed a method to visualize DNA chain elongation on single-stranded ØX174 phage DNA template using gel electrophoresis. As was reported last year, several DNA polymerases (E.coli polI; HeLa cell α-polymerase; Drosophila embryo α-polymerase and avian myeloblastosis virus, AMV, reverse transcriptase) were shown to be unable to elongate nascent chains across pyrimidine dimers, the principal UV-induced lesion in the DNA. We conclude from these results that the suppression of the 3'⟶5' exonuclease may be necessary but is not sufficient for the mutagenic pyrimidine dimer by-pass synthesis (see also contract 224-76-1 BIOB in this laboratory-ref. n°11 and 20 in 1976-80 report)

- Search for a SOS constitutive mutator mutant : genetical and biochemical characterisation of E.coli mutator mutants in the polC gene (Sevastopoulos and Glaser) - A. Brotcorne-Lannoye

E.coli temperature-sensitive mutator mutants mapping in the polC gene (DNA polymerase III, replicase) demonstrates a strong mutator effect, independent of the recA gene which is known to regulate SOS mutagenesis functions. We have found that UV induced mutation frequency is about ten fold higher in these strains than in the wild type parent. However in all the mutants tested, the UV induced mutagenesis is dependent on the recA and lexA gene products. The mutator effects of UV irradiation and the polC mutation appear to have a synergistic effect on mutation frequency, at least for one type of mutation studied (valine resistance). The level of Weigle reactivation of λ phages is the same in all polC mutants tested and is not different from that observed in the wild type parent.

Using the method we developped to detect DNA chain elongation with single stranded ØX174, we have examined crude extracts of the different polC mutator mutants using this system, for the presence of a polymerase activity able to synthesize past pyrimidine dimers. No such activity has, as yet, been detected.

- Evidence for a new pathway for phage DNA repair (G. Maenhaut-Michel and P. Caillet-Fauquet)

If E.coli are grown in the presence of the base analog 2-amino purine (2AP) they exhibit an increase in the ability to reactivate UV or -irradiated λ phages. This reactivation appears to be independent of recA and lexA genes but requires functional mutS gene (mutS bacteria appear to be deficient in mismatch-correction, see contract 224-76-1 BIOB). Therefore it appears that the observed increase in survival of λ phages is not due to the induction of SOS functions which require the expression of recA and lexA genes and which are expressed normally in mutS bacteria. In addition, the increase in viability observed is not accompanied, as in the case of SOS repair, by a major increase in mutagenesis.

2) Mammalian cells

- Tumor promotors, somatic recombination and carcinogenesis (M. Radman, A. Kinsella, P. Jeggo, S. Osborn and A. Mogg) - see also contract 224-76-1 BIOB

Carcinogenesis can be considered as the sequence of initiation (one or several somatic mutations) followed by promotion (not necessarily induced by a DNA damaging agent). This second step can be considered as a mechanism through which (a) recessive mutation(s) become(s) expressed in a diploid cell. This could be the consequence of an aberrant chromosomal segregation event.

The potent tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) has been shown to induce sister chromatid exchanges (SCE's) in cultured cells. A non-promoting TPA derivative (4-O-methyl-TPA) does not induce SCE's. TPA-induced SCE formation is inhibited by inhibitors of tumor promotion, which also inhibit the formation of MNNG-induced chromosomal aberrations. These findings support the idea that chromosomal rearrangement may be involved in carcinogenesis, perhaps as a means to allow the expression of recessive mutations by rendering them homo- or hemizygous. A heterozygous line of V79 chinese hamster cells has been constructed to allow the screening of certain carcinogens and promoters, such as TPA and X-rays, for the ability to induce mitotic recombination or other chromosomal rearrangements leading to the expression of a recessive mutation. Preliminary results suggest that TPA may be positive in this system.

- Induced repair in mammalian cells (J. Cornelis and J. Rommelaere, in collaboration with Dr. J. van der Eb, Leiden, and Dr. Ward, Yale University)

If the problem of induced error prone repair is well documented in bacteria, the case of mammalian cells is much less established. However general reviews have appeared by members of our group (1977, 1980). The problem has been approached with 2 DNA viral systems : the double stranded SV40 and the single stranded autonomous parvoviruses MVM and H-1, the latter giving the possibility to be used on human cells.

- SV40

Preirradiation of confluent monkey host cells with U.V. light increases

1°) the production of irradiated virus
2°) the survival of irradiated virus, and
3°) the reversion rate of normal but not of irradiated tsBC245 virus.

The effects observed were maximal if infection is delayed for 3-4 days after the irradiation of host cells and the increased mutation frequency of normal virus can be explained by the induction in the host cells of transient mutator activity rather than increased number of viral replications performed in the irradiated host cells.

- Autonomous parvoviruses

1) U.V.-irradiation of synchronised host cells in Go induces the activity of effector proteins increasing normal virus production and survival of U.V. irradiated virus ; a bell-shaped kinetics of the induced process is found as for SV40. The comparison of related cell hybrids has demonstrated that these responses can be dissociated from one another and that they are therefore probably accomplished by different proximal effectors.

2) Irradiation of the host cell does not affect prereplicative steps of the viral cycle (penetration, decapsidation, etc...) but increases the capacity of the host cell to convert the single stranded damaged viral DNA into a double stranded replicative form : this could explain the reactivation process.

3) Mutation of a normal or irradiated viral probe can be induced directly (irradiation of the host cell) or indirectly (infecting an intact cell by an irradiated non infectious signal) (SV40 or Parvovirus) showing that damage of a specific host target is not a necessary step.
There is no necessary relationship between the increased survival of irradiated virus and increased mutations. The radiation lesions introduced in the host cell by the non infectious virus apparently constitute (a) signal(s) which induce the expression of a host cell mutagenic function, suggesting that a diffusible effector is implicated in the activation process.

- Immunological detection of DNA damage in cultured mammalian cells

(J. Cornelis)

To understand radiobiological effects and DNA repair it is important to know the fate of the damage and the time during which it persists. Therefore we devoted some time to the preparation of antibodies against radiation damage. Antibodies against pyrimidine dimers were obtained quite successfully and permitted the study by radioautography of their elimination by photoreactivation and enzymatic mechanisms ; however a non negligible proportion of pyrimidine dimers persisted for more than one generation period. It should also be emphasised that the method is able to detect damage obtained with low dosages of radiation of the order of magnitude leaving up to $\pm$ 90 p.c. survivors.
Antibodies against damage caused by γ radiation were less successfull.
Antibodies obtained with irradiated DNA as antigen cannot discriminate between irradiated DNA and single stranded unirradiated DNA. Denatured DNA treated with osmium tetroxide,which should give high amount of pyrimidine glycols, elicites antibodies which were not specific. Therefore another approach should be used like using purified damaged bases as antigens, or increase antibody specificity by preparing them in hybridomes.

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Titre du projet n°3 : Logical, genetic and biochemical analysis of the control of recombination and replication ; mechanism of illegitimate recombination

Chef du projet et collaborateurs scientifiques : R. Thomas, C. Dambly, C. Debouck, D. Espion, Z. Toman, J. Richelle, P. Van Ham, A. Toussaint, M. Faelen, A. Résibois, N. Lefèbvre, F. Van Gijsegem, L. Desmet

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1) Genetic, biochemical and logical analysis of regulatory nets, including those involved in DNA repair, recombination and replication

(C. Dambly, C. Debouck, D. Espion, Z. Toman ; R. Thomas, J. Richelle et P. Van Ham)

As noted in the project, a number of studies in many laboratories deal with the identification of the genes and of the enzymes involved in DNA repair, recombination or replication, and in the assignment of which gene codes for which enzyme subunit ; but although these efforts are entirely justified, it is remarkable to see how little has been done about the structure and operation of the regulatory nets which control expression of these genes.

A major aim of the project was to contribute to filling this gap, with special attention to the circuits involved in the development of temperate bacteriophages, a process in which DNA lesions, recombination and replication are involved. This was achieved by a combined genetic, biochemical and logical approach. The main results are :

a) Identification of the positive control protein cII

The viral gene cII is involved in the decision for or against lysogenization. Its product which had not been isolated so far, was identified as follows. First, it must be mentionned that another gene, cIII, also takes part in the decision, presumably in an indirect way, by stabilizing the cII product. We isolated a bacterial mutant (lycB) in which cIII-deficient mutants of bacteriophage λ can lysogenize at an almost normal rate. Extracts of infected lycB culture contain a protein which is absent in the extracts from wild type E.coli infected cultures. This protein was conclusively shown to be the cII product ; it is absent if the phage carries a nonsense mutation in gene cII, but it reappears if the bacterial host carries a proper nonsense suppressor.

b) Mechanism of the positive control by protein N

The crucial role of protein N in the viral development was demonstrated in this laboratory several years ago. In order to permit a quantitative analysis of the regulation exerted by the N product on early expression, C. Dambly inserted genes from the galactose operon in the "early rightwards operon" of bacteriophage λ ; in this way, the expression of the viral operon can be measured by titrating the activity of enzymes of the galactose metabolism. It was shown that the N product interferes with transcription termination, not with initiation. The recognition site for the N protein is distinct from the promoter and located between the promoter and the first terminator.

c) Epigenetic vs/genetic damages in the stability of immunity (in collaboration with M. Radman).

One of the regulatory loops which take part in the choice between the immune and non-immune states in temperate bacteriophages is a so-called "positive loop" (see section d) involving genes cI (which codes for the λ repressor) and cro. The product of gene cI prevents the expression of gene cro, and vice versa ; this is why the system can persist indefinitely in either of two stable epigenetic states (immune or non-immune), at least when proper mutations prevent the loss of immunity to result in phage development and cell death. C. Dambly, who had connected gal genes with the cro region*, is now able to check the state (immune or non immune) of the cells from their colour on gal indicator. Various agents (radiations or chemicals) can bring about the transition from the immune to the non-immune state, either by a genetic mechanism (mutational inactivation of gene cI) or by an epigenetic mechanism (permanent loss of immunity as a result of a transient inactivation of the repressor).

d) Logical analysis of regulatory nets

A very efficient tool has been developed for the analysis of complex regulatory circuits involving feedback loops. During this 5 year period, this tool has been used first for the analysis of the circuits involved in the decision between lysogenization and the lytic development in temperate bacteriophages. More recently, it was applied by us to problems of normal differentiation and malignant growth in mammalian cells, and by others (e.g. Brygoo thesis, Paris, to epigenetic problems in protozoa).

The method has been greatly refined, and in addition most of its steps have been automatized (by P. Van Ham) in order to permit the treatment of systems with many variables. Conclusions of general interest have emerged. For instance, it has become reasonably clear that the homeostatic type of regulation is dependent on the presence, in the logical structure of the system, of a so-called "negative feedback loop", that is, a loop with an odd number of inhibitoty steps ; in contrast, the diversificative types of regulation (including cell differentiation) require "positive feedback loops", that is, loops with an even number of inhibitory steps.

An EMBO course on the logical analysis of systems has been organized in 1979. The proceedings have been published in "Lecture notes in Biomathematics" under the title "Kinetic logic" (R. Thomas, editor, 507 pp.)

2) Studies on the mechanism of illegitimate recombination

(A. Toussaint, M. Faelen, A. Résibois, N. Lefèbvre, F. Van Gijsegem and L. Desmet)

A sizeable fraction of the genetic damages induced by ionizing radiations consists of non-homologous ("illegitimate") exchanges (translocations, deletions, etc...). So far, the mechanisms of illegitimate recombination had remained completely unknown. During this contract they have been studied with a remarkable success, using the "mutator phage" Mu-1.

a) Mu-mediated inversions, duplications and deletions

As mentionned in the project, A. Toussaint and M. Faelen had shown previously that phage Mu can efficiently connect two DNA sequences devoid of any homology, and promote transposition between unrelated genetic elements. A model proposed to account for these results predicted that

*these strains are distinct from those mentionned in section 1, b.

in addition phage Mu should also promote inversions, deletions and duplications. Each of these predictions was verified experimentally. The occurrence of inversions was demonstrated in an especially elegant way ; advantage was taken of the idea that when an Hfr strain undergoes an inversion of the region carrying the integrated F factor, the direction of the transfer of the chromosome is reversed.

b) Genes involved in Mu-mediated chromosomal rearrangements ; miniMu, miniMu-mediated transduction, miniMu-duction

The Mu group soon reached the conclusion that the elements absolutely required for the chromosomal rearrangements are the terminal sequences of Mu DNA and the product of the viral gene A. If this is correct, one should be able to isolate deleted Mu prophages which have kept only the termini and gene A and are nevertheless still able to promote chromosomal rearrangements. Such deleted prophages ("miniMu") were indeed obtained. Gene A itself may even be deleted, if one provides the A product in trans.

The next step consisted in obtaining miniMu virions. This can be done by inducing a strain carrying both an inducible miniMu and an inducible Mu; about 10% of the virions contain a miniMu genome. Since a Mu virion contains a constant amount of DNA - the Mu genome itself flanked by two sequences of bacterial DNA, a very short one and a longer one, it was anticipated that the miniMu DNA, in which the Mu genome itself is very short, would contain a very long sequence of bacterial DNA. The idea was demonstrated by electron microscopy by A. Résibois (very long "split ends") and resulted in the discovery of generalized transduction by miniMu. In addition a new type of transduction was discovered at the same time : "miniMuduction", a process which occurs when the piece of DNA included in a virion happens to contain two miniMu of the same orientation, separated by a section of bacterial DNA. This piece of DNA behaves in fact as a transposon which would be encapsidated.

c) D108, mini D108

D108 is a phage related to Mu, but of a different specificity of immunity. We could show that it promotes the same chromosomal rearrangements as Mu, and isolated mini D108, which behave essentially as miniMu. The transposition enzymes are not interchangeable in the two systems.

d) Mechanism of replication of Mu

In collaboration with B. Waggoner and M. Pato, we used the deleted derivatives of Mu to study the mechanism of replication of the phage. Earlier observations indicated that two viral functions, the products of genes A and B, are required. Our recent results strongly suggest that a third viral gene (arm) located between B and C, is involved.

e) Mini-Mu induced mobilization of the host chromosome by plasmid RP4 ; intergeneric rearrangements.

We wanted to extend to bacterial species other than E.coli the method of mini-Mu induced mobilization of the chromosome, and inclusion of chromosomal segments into transmissible plasmids. This goal was reached by integrating a mini-Mu into RP4, a transmissible plasmid with a wide host range. In contrast with RP4 itself, RP4::mini-Mu induces the transfer of the host chromosome at a high rate (10^{-4}) and generates "RP4-primes" (that is, RP4 which carry a segment of the host chromosome) at frequencies of the order of 5×10^{-7} for a given marker. RP4::mini-Mu has been introduced into strains of Salmonella typhimurium, Klebsiella pneumoniae and

Proteus mirabilis. These strains were crossed with a strain of E.coli, and in each case, exconjugants carrying RP4-primes were found at frequencies between 10^{-5} and 10^{-7} : thus, genes from the three alien genera could be transferred in E.coli, in which they are expressed.

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Titres du projet n° 4.

1. Radiosensitivity of the first differentiation events in the mouse (H. Alexandre)
2. - Radiation responses of somatic cell hybrids (F. Zampetti-Bosseler, S. Limbosch, J.M. Vos, J. Cornelis and J. Rommelaere).
   - Investigation of X-ray-induced cytological damage in relation to cell lethality (F. Zampetti-Bosseler and D. Scott, Paterson Laboratories, Manchester, U.K.).
3. Control of growth and of genetic expression in cultured mammalian cells (C. Szpirer, J. Szpirer, A. Poliard, D. Saggioro).

Chef du projet et collaborateurs scientifiques : J. Brachet, H. Alexandre, F. Zampetti, S. Limbosch, C. Szpirer et J. Szpirer.

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1. We have shown that oocyte maturation can be divided into two steps according to their radiosensitivity : the radioresistant one corresponding to the germinal vesicle breakdown and the highly radiosensitive one to the first polar body extrusion. Moreover, it was interesting to note that intermediate doses of X-rays (450R) exert effects similar to that of cycloheximide and may be therefore tentatively correlated with the inhibition of the microtubule assembly at metaphase I. Lower doses, which do not affect the apparent normal resumption of meiosis, induce dose-proportional chromatid deletions, resulting in severe teratogenic risks.

   The relative radiosensitivity of the uridine, deoxyuridine and thymidine incorporation into DNA at different preimplantation stages of the mouse embryo, has clearly indicated that both thymidilate synthetase and ribonucleotide reductase activities are more radiosensitive than the DNA polymerase activity, at all stages studied. Moreover, these pyrimidine metabolic pathways radiosensitivities decrease progressively from the 2-cell to the late morula-early blastocyst stage, as a result of the ontogeny of involved enzymes.

   On the base of irradiation experiments, cytological examinations, autoradiography and in vitro inner cell mass differentiation, we proposed a general explanation of the various kinds of defects caused by irradiation of preimplantation embryos : no selective effect of irradiation on inner cells of the early morula can be considered; preimplantation abortion is due to a very early cell killing, early postimplantation abortion is due to an abnormal low cell number of the nascent blastocysts and, finally, teratogenic risk should be due to chromosomal abnormalities in almost normal sized blastocysts (very low percentage).

   In close relation with this explanation, we studied in more details the factors determining cavitation in mouse morula mainly in two experimental conditions : metabolic quiescence induced by a transient inhibition of polyamine synthesis and, specific inhibition of nuclear DNA replication by aphidicolin. We clearly confirmed that the blastocyst formation is unrelated to the cell number and we showed that both inhibitory systems are, in that point of view, radiomimetic. This enabled us to study the differentiation of blastocysts made of a low cell number, without embryonic derivatives. However, cytodensitometric measurements have shown that the nucleo-cytoplasmic ratio seems to be an important prerequisite if not a trigger for cavitation.

2. Somatic cell hybrids have been used as a tool to elucidate factors and mechanisms involved in mammalian cell radioresistance. The main conclusions of these studies are that resistance to X-rays, as well as UV light, may behave in a dominant way and that the survival capacity of mammalian cells after X- or UV- irradiation is not related to chromosome number. We have also observed that cell hybridization may generate, in some cases, valuable systems for repair studies such as hybrids between L5178Y mouse lymphoma cells and A9 mouse fibroblasts. Indeed, these hybrids exhibit an increase in resistance to either X-rays and UV (hybrid clone 3) or X-ray alone (hybrid clones 1 and 2) compared to parental cells. Several possibilities have been examined to understand the UV response of hybrid clone 3. It was found that the increased resistance of this hybrid was not related to changes in cell size and growth rate; nor was it related to an enhanced recovery of the capacity to synthesize high molecular weight DNA after UV-irradiation (tested indirectly by the use of caffeine) and or to an altered regulation of an inducible repair pathway (studied by UV-Reactivation of Minute-Virus-of-Mice). However, hybrid clone 3 was found to be distinguishable from more UV-sensitive parental and hybrid (clones 1 and 2) cells by its greater capacity for UV-induced unscheduled DNA synthesis. Hence, it is possible that hybrid clone 3 would have a better excision ability, which could explain its resistance to UV. On the other hand, an interesting finding has been made on L5178Y x A9 hybrids with regard to the mechanisms of UV-induced repair pathways in eukaryotic cells. We have demonstrated that the expression of Enhanced Capacity (EC) (i.e. the UV-enhanced ability of irradiated cells to replicate intact virus) can be dissociated from that of Enhanced Reactivation (ER) (i.e. the higher survival of irradiated virus in preirradiated cells). Moreover, we have shown that the expression of both EC and ER required de novo protein synthesis shortly after cell irradiation. Finally, hybrid clones 1 and 2, which display EC but are devoid of ER activity, show promise as systems for studying the correlation between EC and mutagenesis.

   In an earlier work, we showed that X-ray-sensitive mouse lymphoma cells sustain more chromosome damage, mitotic delay and spindle defects than X-ray-resistant mouse lymphoma cells. We suggested that a) chromosome aberrations contribute much more to lethality than spindle defects and b) that DNA lesions are less effectively repaired in the sensitive cells and give rise to more G_2 mitotic delay and chromosome aberrations. To test these hypotheses, we then investigated the magnitude of these various end-points in human diploid fibroblasts with reported differential sensitivity to ionising radiation (i.e. normal donors and patients with ataxia telangiectasia and retinoblastoma). Our results on human fibroblasts support the first hypothesis since we observed a positive correlation between chromosome aberration frequencies and cell killing, and no induced spindle defects. Our second hypothesis is however not substantiated since X-ray-sensitive fibroblasts from the ataxia patient suffered less mitotic delay than cells from normal donors. A common lesion for mitotic delay and chromosome aberrations can still be assumed by adopting the hypothesis of Painter and Young (1980) that the defect in ataxia cells is not in repair but in a failure of DNA damage to initiate mitotic delay.
   In contrast to other workers, we found the retinoblastoma cells to be of normal radiation sensitivity (cell killing and aberrations).

3. We have analyzed various types of hybrid cells derived from hepatoma cells. We have shown that the synthesis of some serum proteins (albumin, α-foetoprotein) is switched off in hybrids formed between these hepatoma cells and fibroblasts (either tetraploid fibroblasts from permanent cell lines, or normal diploid fibroblasts). Such hybrids do not contain detectable sequence of albumin messenger RNA. "Extinction" thus seeems to be due to regulatory molecules which

negatively operate on the hepatoma genome, possibly at the level of gene transcription. Unlike hepatoma x fibroblast hybrids, hybrids formed between hepatoma cells and normal adult hepatocytes (which do not produce AFP) retain the production of α- foetoprotein coded for by the hepatoma genome but do not exhibit activation of the production of α-foetoprotein coded for by the normal hepatocyte genome. This lack of interaction between the two genomes indicate that cis-acting control mechanisms also operate in differentiated cells. The hepatoma x hepatocyte hybrids (called "hepatocyte hybridomas") secrete serum proteins (such as albumin, transferrin etc...) coded for by the hepatocyte genome : immortalization of the production of serum proteins is thus achieved in such hybrids. On the other hand, these hybrids segregate rat chromosomes and can be used for mapping studies.

The study of hepatoma cell x normal diploid fibroblast hybrids has also shown that the transformed phenotype of the hepatoma parent is suppressed after fusion with the normal partner. Similarly, hybrids formed between two fibroblasts, a normal one, and a (spontaneously) transformed one exhibit a normal phenotype, indicating that correction of the transformed phenotype is not due to peculiar interactions ("extinction" of differentiated functions) between the genomes of cells derived from different tissues. Different X-ray transformed fibroblast clones have been isolated and we have shown that unlike spontaneously transformed fibroblasts, these X-ray transformed fibroblasts possess a matrix of pericellular fibronectin; a lack of fibronectin matrix is thus not a periquisite for the expression of transformation, especially of X-ray induced transformation. The expression of transformation has also been studied in hybrids derived from X-ray and found to be suppressed

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TITRE DU PROJET N° 5 : Radiosensitivity of the immune system, graft versus host disease and suppressor T cells.

CHEF DU PROJET et COLLABORATEURS SCIENTIFIQUES : J. URBAIN, M. MOSER, C. BRUYNS, A. VAN ACKER, G.URBAIN-VANSANTEN, N.TASIAUX et P. VAN DE WALLE.

The results which we have obtained during this research program should be understood in the frame of new developing concepts of immunology. We shall first introduce these concepts.
During the last years, the classical concepts of immunology have been challenged and replaced by new ideas, strongly supported by a bunch of hard data. The new facts and the new concepts establish the need for a revision of the effects of low dose irradiation on immune response. A short summary of the new intellectual framework is necessary before discussing the effects of mild irradiation. A more complete survey can be found in a recent review written by J. Urbain, C. Wuilmart and P.A. Cazenave (Contemporary Topics in Molecular Immunology 1980).

a. Old Immunology

In old immunology, the classical clonal selection theory (Jerne, Lederberg, Burnet) was the main paradigm among immunologists. Essentially, the immune system was viewed as a library of small uncoupled immune systems (lymphocyte clones). Each clone is bearing immunoglobulin receptors of one specificity (two V genes are actually expressed (V_H and V_L). When antigen is introduced in the system, it selects clones bearing receptors which happen to fit with antigen and promotes the amplification (proliferation and differentiation) of those precommitted clones. Each clone was believed to respond independently of the other clones. This was essentially a "prokaryotic" or "unicellular" view of the immune system. The proliferation needed for an immune response was an evident target for irradiation.

b. The new visage of immunology

We can only give here a very rough outline of the new concepts of immunology. It is now almost certain that the immune system is a complex cellular and idiotypic network. Clones are not independent entities but each lymphocyte gives and receives signals from some other lymphocytes. The connectance within the network forms the basis of important immunoregulatory circuits of the immune systems.

1. In the case of T dependent antigens (this corresponds in fact to all antigens which are not polyclonal B or T cell activators), a B lymphocyte never transforms into an antibody producing cells unless it receives some differentiation signals from T helper lymphocytes.

2) The world of T lymphocytes is subdivided into subsets committed and performing different physiological functions. T helpers promote the differentiation of B cells and T suppressor lymphocytes are induced when the number of helpers reach a certain threshold of concentration In other words, induction of an immune response leads automatically to activation of a feedback loop (suppressor loop) which dampens the ongoing immune response. Regulation of an immune response does not depend only on external signals (antigen) but is also due to signals generated within the immune system. Dampening of the immune response by suppressor T lymphocytes leads to a decrease in binding affinity. These suppressor T lymphocytes are especially radiosensitive during certain stages on this differentiation (even to low dose irradiation) and they develop less efficiently in irradiated animals. It should be stressed that even doses as low as 25 Rads have a significant effect on antibody affinity (see also below).

3) T lymphocytes use a dual recognition system : one immune receptor (V anti-X) does recognize antigen or idiotypic specificities and a physiological receptor (R anti-H) does recognize membrane self markers encoded within the major histocompatibility complex. The two receptors are functionnaly linked. The physiological receptor of T lymphocytes is not genetically programmed but is selected or acquired during ontogeny of the T lymphocyte, within the thymus. The thymic epithelium seems to dictate the restrictive specificity.

4) The immune system is a functional idiotypic network. Idiotypic interactions can take place within the system. For example, helper T cells bearing autoantiidiotypic receptors can promote the differentiation of B cells bearing receptors displaying complementary idiotypic specificities.

5) The immune system is not a collection of clones waiting for an antigen but an enormous turnover takes place within the immune system. This means that an immune response is not due simply to activation of already present clones but is also due to "recruitment" of newly differentiation stem cells which constantly replenish the immune system. This property has dramatic consequences in the field of radiobiology of the immune system.

6) Suppressor T lymphocytes are not only responsible for feedback suppression of an ongoing immune response but determine also the size of the repertoire which is accessible to one antigen. Suppression is dominant in the immune system. Silent clones are not insignificant minorities but are kept under active suppression. Since suppressor T lymphocytes are especially radiosensitive, this fact has also obvious consequences with considering the effects of low dose irradiation on the immune system. It is beyond the scope of the short introduction to give an unified picture of the new concept. Such views are discussed in several publications of this laboratory.

Suppressor T lymphocytes : radiosensitivity, function and induction during the immune response.

When spleen cells of immune mice are transferred together with antigen into normal syngeneic recipients, the differentiation and expression of memory cells is counteracted by suppressor T cells which develop in the recipient.
This has been repeatedly observed with different antigenic systems (TMV, Hemocyanine, DNP-OVA, arsonate...).
This phenomenon which has been called the isogenic barrier is an exception to the classical laws of transplantation : since donor and recipient mice belong to the same inbred strain. Such an inhibition is not observed in nude mice who lack functional T cells. The isogenic barrier can be prevented if recipient mice are irradiated at low dose (150R) before all transfer, or if antigen is injected several times.
The same inhibition phenomenon can be studied in vitro by coculturing immune cells and non immune cells.
It is therefore clear that this phenomenon represents a state of transient inactivation which is clearly different from tolerance.
The results which have been obtained so far suggest that the isogenic barrier is in fact an amplification of the normal feedback mechanism which occurs in any normal immune response. This feedback mechanism seems to be associated with a decrease in binding affinity perhaps partly due to lymphocytes bearing autoantiidiotypic receptors.
The role of suppressor T lymphocytes in enhancing tumor growth has been evaluated. Low dose irradiation (150R) at 6 days after tumor implantation leads to an important reduction of tumor growth. Moreover, isolation of suppressor T lymphocytes which appear after tumor inoculation and preimmunization of syngeneic recipients with these suppressor T lymphocytes results in a state of specific immunity against the tumor.

Protection of normal individuals and radiation chimaeras by high affinity antibodies.

The protection of individuals against infection relies on the efficiency of the immune system. This efficiency depends on the affinity of antibodies and T cell receptors for antigen. Irradiated rabbits grafted with allogeneic lymph node, spleen and bone marrow cells from donor rabbits hyperimmunized against TMV synthesize immediately high affinity antibodies, displaying recipient allotypic specificities. Recipient rabbits from non immune donors synthesize antibodies of lower affinity. However, in radiation chimaeras, the average affinity was always greater than in normal animals immunized against the same schedule. This higher average binding affinity is probably due to the partial absence of suppressor T lymphocytes, some of which recognize autoantiidiotypic receptors.

It seems clear that the average affinity of antibodies depends on the control exerted by different T subpopulations, characterized by different radiosensitivities.

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Contractor : Trinity College, Dublin

Contract No. : 190-76 BIOEIR

Head of research team(s) : Professor F.G.A. Winder

General subject of Contract : Enzymology of repair of radiation damage to DNA

Results of Project No. : 1

Head of project and scientific staff: Professor F.G.A. Winder, Dr. M.J. Carroll, Mr. W. Maxwell, Miss S. Hemmens, Miss R. Harvey and Miss M. Coakley.

Title of Project: Enzymology of repair of radiation damage to DNA.

We have purified and investigated two proteins, of M_r 70 000 and 47 000, which separately bind to DNA polymerase I of Mycobacterium smegmatis to form loose complexes with altered affinity for single-stranded DNA and modified catalytic properties. However, the biological significance of the formation of these complexes is not yet clear. Some evidence has been found that DNA polymerase I of Escherichia coli can also complex loosely with other proteins to give forms with altered properties.

We have tried to relate our findings on the deoxyribonucleases of Aspergillus nidulans to findings with these enzymes in mammalian cells, but this has made clear the severe shortage of information on the neutral endodeoxyribonucleases of mammalian tissues other than the bovine pancreas. We have started an investigation of these enzymes in different tissues of several mammals.

Studies on the toxicity of tritiated compounds for the mouse embryo in vitro have continued in collaboration with the group of Dr. F. Campagnari. Results have generally been compatible with the concept that toxicity in this system is related to the extent of incorporation into DNA or histones. However, some anomalous results have been obtained, particularly with [^{3}H]tryptophan.

Further work on the DNA polymerase activity of Mycobacterium smegmatis has led to the revision of some of the conclusions contained in the 1979 report. DNA polymerase I activity from this organism has been resolved into three peaks with different affinities for single-stranded DNA. All active fractions contained a M_r 109 000 polypeptide, identical in size with DNA polymerase I of E. coli. Complement-fixation assays have shown that this polypeptide reacted well with antibody to E. coli DNA polymerase I. Material from the peak of activity with lowest affinity for DNA could be resolved into two regions by gel filtration: one of these contained a large amount of a dimer of the 109 000 polypeptide, devoid of polymerase activity, while the second contained the 109 000 polypeptide in loose binding with a 47 000 polypeptide, showing specific polymerase activity higher than that reported for E. coli polymerase I. Material from the peak with highest affinity for DNA contained the 109 000 polypeptide in loose binding with a 70 000 polypeptide, again showing very high specific polymerase activity. Examination of material from the peak with intermediate affinity showed a more complex situation in that region. Thus, the results provided evidence for the presence of at least two complexes of the DNA polymerase polypeptide with other proteins, in addition to a dimer of the polymerase which retained DNA-binding capacity without polymerase activity.

Ion-exchange cellulose chromatography of the fractions containing the complexes separated the 109 000, 70 000 and 47 000 polypeptides and gave each in homogeneous form. This confirmed that the complexes of the polymerase with the other polypeptides are fairly loose ones. Neither the 70 000 nor the 47 000 polypeptides showed polymerase activity, nor did their presence substantially affect the level of polymerase activity of the 109 000 polypeptide with DNA as template, though both affected the kinetics of its action, particularly with poly d(AT) as template. Both the 47 000 and the 70 000 polypeptides possessed some nuclease activity with native DNA, but it was inhibited by deoxyribonucleoside triphosphates. Both increased the 5'→3' nuclease activity shown by the 109 000 polypeptide, but their effects on its 3'→5'-nuclease activity were slight. Their effects on accuracy of incorporation have varied, and need further investigation. Preliminary results indicate that the increased polymerase activity which follows DNA damage in this organism is accompanied by an increased amount of the 70 000 polypeptide.

Similar investigations with extracts of Escherichia coli have shown that, in these also, the polymerase I activity could be resolved into at least two forms with different affinities for DNA and somewhat different catalytic properties. However, the properties of the forms differ from those found with M. smegmatis and need further investigation.

Our previous investigations on the DNAases of Aspergillus nidulans had shown that this organism contains two acid DNAases similar to mammalian DNAase II, a DNAase rather tightly bound to chromatin similar to the Ca, Mg-endonuclease of mammalian chromatin, and two DNAases similar to mammalian DNAase I. One of the two latter DNAases has an endogenous inhibitor similar to actin, the endogenous inhibitor of DNAase I. One of the purposes of our characterization of the A. nidulans DNAases was the hope that their comparison with mammalian DNaases would facilitate the recognition of those involved in universal functions, such as replication and repair, as distinct from functions peculiar to the way of life of the organism concerned. However, knowledge of mammalian endodeoxyribonucleases, apart from a few such as bovine pancreatic DNAase, spleen DNAase II and some of the enzymes specific for damaged DNA, is very fragmentary, so that we have had to initiate our own study of mammalian DNAases, using several tissues from each of four unrelated mammals. This work is at too early a stage for general conclusions.

Studies on the toxicity of tritiated compounds for the growth of the mouse embryo in vitro have continued in collaboration with the group of Dr. F. Campagnari. Results with tritiated nucleosides, amino acids and water have generally been compatible with the concept that the relative toxicity of such compounds for the growing embryo is related mainly to the extent of incorporation of these compounds into DNA or histones. Inhibition of blastula formation required incorporation into DNA of the order of 10 dpm per embryo, while inhibition of blastula hatching required substantially less. Anomalous results were obtained with [^{3}H]tryptophan, which was more toxic than expected and prevented embryos from developing beyond the 2-cell stage.

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Contractor : University College, Galway, Ireland.

Contract nr. : 189 - 76 - 1 BIO EIR
Head of the research team : Professor James A. Houghton

General subject of contracts : Studies on the effects of radiation on the blue-green algae.

Title of the project nr.1. Studies on the effects of radiation on the blue-green algae.

Head of project and scientific staff : Professor James A. Houghton, Mr. Philip O'Brien, Mr. C. Geoghegan, Mr. P. Lanham, Ms. V. Murphy and Professor L.K. Dunican and Dr. Williams.

The cyanobacteria or blue-green algae are a unique group of organisms and they constitute the largest, most diverse and widely distributed group of photosynthetic prokaryotes. They contain a photosynthetic apparatus which is similar in structural, functional and molecular respects to the system found in eukaryotic chloroplasts. However, striking similarities in cellular organization, cell division and cell wall structure between cyanobacteria and bacteria clearly indicate that the two groups are very closely related.
The cyanobacteria thus contain, within a prokaryotic cell, a photosynthetic mechanism which resembles in most respects that found in eukaryotic chloroplasts. Indeed, it has been suggested that the chloroplasts of higher plants evolved from an endosymbiotic cyanobacterial cell which developed within a colourless cell and carried out its photosynthetic activities. The cyanobacteria are consequently of immense phylogenetic significance and play a key role in our understanding of biological evolution. Furthermore, the cyanobacteria are a very ancient group and they evolved before the development of an oxygen atmosphere on Earth and it was largely the photosynthetic activities of these organisms that produced the Earth's oxygen atmosphere. It has thus been argued that the cyanobacteria evolved at a time when there was little protection from solar irradiation and their survival was due to the development of efficient radiation repair processes. Similar systems are present in many different bacterial and eukaryotic cells. Studies on cyanobacterial repair, therefore, provide information on the development and evolution of radiation repair mechanisms in both prokaryotic and eukaryotic systems.

In this project the effects of radiation on a unicellular cyanobacterium Gloeocapsa alpicola were investigated and the radiation repair systems studied. In the early stages of the project UV survival was examined and its effects were found to differ markedly depending on whether a far (254 nm) or near (300-350 nm) UV source was used for cell irradiation. Near UV had a much greater inhibitory on cell survival and photoreactivation was inefficient. Carotenoid pigments played an important role in protecting the cells against near UV damage since reduction in carotenoid levels reduced the near UV survival rate. The photosynthetic rate of G. alpicola dropped sharply after cellular exposure to UV irradiation. Near UV had a greater inhibitory effect on photosynthesis than far UV. Following irradiation, far UV irradiated cells quickly recovered their pre-irradiation rate whereas near UV irradiated cells were much slower to recover their photosynthetic activity. The results of this section of the project indicated that near UV had a damaging effect on the photosynthetic system of the cell and carotenoids played a protective role. The major effect of far UV was on the DNA of the cell and this was investigated in great detail. Studies on the repair of far ultraviolet light (FUV) damaged DNA have revealed that G. alpicola has an extremely efficient photoreactivation system. Exposure to blue light for 60 min immediately after irradiation restored viability to pre-irradiation levels even when viability had been reduced to 0.001%. Work has continued on the isolation, purification and characterization of the photoreactivating enzyme and the technique of flash photolysis has been used to determine the number of enzyme molecules per cell and the kinetics of the enzyme/dimer complex.

In the cyanobacteria, whilst there is evidence from a number of species for a photoreactivation system, no conclusive evidence of dark repair has been presented. In this project, the presence of an efficient pre-replication dark repair system in G. alpicola has been successfully demonstrated. The evidence for this system was initially derived from studies on the loss of photoreversibility in dark liquid held (DLH) cultures. After a delay of 4h, the ability to photoreverse lethal FUV lesions was gradually lost during DLH in non-growth conditions. Unirradiated controls and non-photoreactivated, FUV-irradiated cultures maintained constant viability during this holding period. Split-dose experiments indicated that the activity of the photoreactivating enzyme did not decrease during DLH and so it was concluded that the loss of photoreversibility was due to competition for the FUV induced thymine dimers by the photoreactivating enzyme and an excision repair endonuclease. This was substantiated by the induction of a delay in the onset of loss of photoreversibility when the excision repair inhibitors caffeine, coumarin and pyronin Y were incorporated into the

DLH medium. This indirect evidence prompted an investigation into direct biochemical proof of the presence of an excision repair system in G. alpicola by the isolation of the enzyme involved in the first step of the pathway. Having developed a suitable lysis procedure for G.alpicola, a UV-endonuclease with activity towards FUV-irradiated lambda DNA and no activity towards unirradiated DNA was isolated from a crude lysate using ammonium sulphate precipitation and ion exchange procedures in the purification. The UV-endonuclease was found to have a molecular weight between 15,000 and 18,000 when estimated on Sephadex G-75. The enzyme had no absolute requirement for Mg^{++} in the reaction mixture but ATP was essential for maximum activity. Caffeine was found to decrease the activity of the enzyme supporting the view that the delay in the loss of photoreversibility by caffeine is due to interference with the excision repair system. DNA repair systems were also investigated by analysing the induction and removal of radioactively labelled pyrimidine dimers. As cyanobacteria are obligately photoautotrophic, difficulties were encountered in the labelling of the genetic material but this was successfully achieved. Pyrimidine dimers were induced in a fluence dependent manner. Dimers were not detected in irradiated cells after 15h incubation in non-photoreactivating conditions. The efficiency of the photoreactivation system of G. alpicola was also demonstrated by the elimination of pyrimidine dimers from irradiated DNA by exposure to blue light. Studies have also continued on the effects of ionizing radiation by the measurement of the production and repair of strand breakage. Radiation resistant and sensitive mutants have been isolated and their radiation repair processes are being studied to establish the biochemical and genetic control of these processes.

During this research programme, investigations have been underway on the genetic control of radiation repair in the cyanobacteria. Very little information was available on the genetics of G. alpicola. A detailed investigation of mutagenesis has been completed and has indicated that whilst G. alpicola is more sensitive than E. coli to the lethal effects of chemical mutagens, it is more resistant to ultraviolet light and ionizing radiations. Ultraviolet light was found to be the most efficient mutagen for G. alpicola provided that the treated cells were rigorously protected from exposure to light of photoreactivating wavelengths. In order to study the effects of radiation on genetic exchange in cyanobacteria, the process of transformation has been investigated. The first example of this process in G. alpicola has been reported and intergeneric transformation between G. alpicola and A. nidulans has been demonstrated for the first time. Cyanobacterial plasmids are also being studied and their role in radiation repair processes are being investigated.

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Contractor : University College, Galway, Ireland.

Contract nr. : 247 - 77 - I BIO EIR
Head of the research team : Professor James A. Houghton

General subject of contracts : Studies on the effects of radiation on satellite associations of human chromosomes.

Title of the project nr. 1. Studies on the effects of radiation on satellite associations of human chromosomes.

Head of project and scientific staff : Professor James A. Houghton, Ms. P. O'Grady, Ms. V. Murphy, Ms. M. Bedard, Ms. F. Dunne and Dr. S.E. Houghton, Dr. Khokhar and Mr. Min.

In metaphase preparations of human chromosomes, the acrocentric chromosomes of groups D and G may often be observed associated together with their satellites directed towards each other and in close proximity. This phenomenon of satellite association is thought to be the result of the involvement of the acrocentric chromosomes in nucleolus formation. In man, the genes coding for 18s and 28s ribosomal RNA are located in the nucleolus organizer regions of the satellited acrocentric chromosomes. As the nucleoli are formed after telophase, they coalesce together to form the common nucleolus of interphase. When the cell starts to divide, the nucleolus disappears but it is thought that the viscous nucleolar material tends to keep the chromosome arrangement held together and this is observed as satellite association in metaphase. The frequency of satellite association varies within the population and it has been suggested that a tendency towards increased satellite association may be an inherited trait. Other factors have also been suggested to affect the frequency of satellite association including age, sex, chemical exposure and chromosome culture technique. It has been repeatedly suggested that satellite association may be one of the determining factors in the causation of chromosome abnormality in man. It has been argued that satellite association may keep the acrocentric chromosomes together during mitosis and meiosis and could thus lead to chromosome non-disjunction. Furthermore, mechanical stretching of the associated satellites could increase the risk of breakage and lead to translocation. It is therefore suggested that the participation of the acrocentrics in association increases their vulnerability to chromosome non-disjunction, DNA exchange and

rearrangement. These hypotheses have stimulated investigation of satellite association in normal, Down's syndrome and other chromosomally abnormal populations. Since non-disjunction is usually a pre-zygotic event, the parents of aneuploid children have also been studied to determine if they have abnormal frequencies of satellite association in their cells. Some studies have shown significant increases in association in Down's syndrome children and their parents. Other studies have failed to show significant differences between Down's patients, their relatives or controls. The role of satellite association in the origin of non-disjunction is still, therefore, unresolved.

In recent years it has become possible to study the activity of the nucleolus organizer regions (NOR) using the techniques of in situ hybridisation and silver staining. In the latter technique, only those NORs which were transcriptionally active in the previous interphase stain with silver. It is now clear that only acrocentric chromosomes with active NORs participate in satellite association.

In this contract, the effects of radiation on satellite association and NOR activity have been investigated in order to establish whether radiation may play a role in the induction of satellite association-related chromosomal non-disjunction in man.

In the first stage of the contract, the effects of a range of radiation sources; including UV, gamma rays, X-rays, electrons, neutrons; on the frequency and composition of satellite association complexes in cells from normal males and females of various ages, Down's syndrome males and females, the parents of children with trisomy and translocation Down's syndrome and patients with other chromosomal aberrations were studied. The data were analysed to determine whether radiation had any effects on the total numbers of chromosomes participating in association, the numbers of different individual chromosomes participating, the number of association complexes per cell and the number of chromosomes involved in each association complex. The distribution of the numbers of complexes in the cells was also determined. The results of this study clearly show that radiation has no significant effects on satellite association in human somatic cells. In situ hybridization and silver staining studies on NOR activities were also carried out to determine whether it was possibleto detect any effects of radiation on NOR activity which might then be expected to lead to changes in satellite association frequency. Using these techniques it was not possible to detect any changes. To determine whether the techniques being investigated were sufficently sensitive to detect small,

radiation-induced changes in NOR activity, studies were undertaken on the effects of chemicals known to have an effect on secondary constrictions or rRNA synthesis. These included actinomycin D, mitomycin C, caffeine, hydroxyurea, 5-bromodeoxyuridine, chloramphenicol, 5-fluorodeoxyuridine and 2-deoxyadenosine. Actinomycin D inhibits rRNA synthesis and has been shown to reduce the silver stain ability of nucleoli in human fibroblasts. However, in this study it was found that whilst it caused morphological changes in the nucleoli, no consistent changes in NOR activity or satellite association patterns were detected. No effects could be detected using these other inhibitors of rRNA synthesis. Similarly, seminalplasmin, a protein recently isolated from human semen and known to inhibit rRNA synthesis in microorganisms, was investigated. No observable effects on NOR activity or satellite association frequencies in normal or chromosomally abnormal cells were identified. The effects of other factors have also been investigated. In particular, it has been suggested that satellite association frequency decreases with the numbers of cell cycles in culture. Using cells taken from normal and chromosomally abnormal individuals, the relationship between satellite association frequency, NOR activity and the number of nucleoli in interphase cells has been established following irradiation Cultured cells in first and subsequent mitoses were determined using the BUDR technique and observations suggest that thelength of time that cells spend in interphase influenced the association of the NOR chromosomes. Radiation had no significant effect on this relationship.

Further studies have been carried out to determine whether any regulatory mechanisms were present to maintain the level of NOR activity, the control of NOR activity was studied in patients with abnormal numbers of NORs as a result of acrocentric chromosome aberration. The results clearly indicated the absence of a mechanism for stringent control of NOR activity.

The investigations carried out during the course of this contract achieved their aims in that they clearly demonstrated that the effects of radiation on satellite association and its consequent effects in increasing the risk of chromosome non-disjunction may be discounted in assessing the risks of irradiation in man.

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Contractors:	1. Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands 2. Institute of Radiation Genetics and Chemical Mutagenesis, University of Leiden, The Netherlands 3. The Medical Biological Laboratory, TNO, Rijswijk, The Netherlands. 4. MRC Cell Mutation Unit, University of Sussex, Brighton, United Kingdom
Contract Nos.:	196-76 BION 192-76-1 BION 200-76 BION 166-76-1 BIO UK
Head of Research Teams:	Professor D. Bootsma, Rotterdam, The Netherlands Professor B. A. Bridges, Sussex, United Kingdom
General subject of contract:	To identify and characterize variant strains of mammalian cells deficient in repair of DNA damage

Six years ago we set ourselves the task of attempting to understand how human beings deal with DNA damage produced by agents such as ionizing radiation both at the cellular and biochemical levels. Our strategy was to isolate and characterize mammalian cell variants showing differences in sensitivity that might be due to deficiencies in DNA repair. The collaboration between the contracting laboratories has resulted in the successful identification of several mutations. The first human mutation (ataxia-telangiectasia) conferring cellular sensitivity to ionizing radiation has been established. Fibroblasts cultured from patients with ataxia-telangiectasia show a D_0 of 57 $\pm$ 15 rads compared with a control mean of 126 rads. In addition, intermediate radiosensitivities are shown by a number of hereditary human conditions such as Hutchinson Gilford progeria, familial retinoblastoma, Fanconi's anaemia, at least some Huntington's chorea, Blackfan-Diamond disease (pure red cell aplasia) and some ataxia-telangiectasia heterozygotes. Human variants sensitive to other DNA damaging agents have also been identified and studied since experience with lower organisms has shown that deficiencies in different steps in the pathways of DNA repair may manifest themselves as hypersensitivity to different types of mutagen. Repair of radiation damage, therefore, cannot profitably be considered except in the context of DNA repair as a whole. Many cell strains with enhanced ultraviolet light sensitivity have, for example, been studied, including patient 11961, Cockayne's syndrome, and nine phenotypically or genotypically

distinct types of xeroderma pigmentosum (XP). Of the latter, the seventh complementation group of excision-deficient XP was identified and characterized in our laboratories. Work has continued on the characterization of variant XPs that appear to possess normal excision repair but are defective in DNA synthesis after UV irradiation.

We have also isolated radiation-sensitive mutants of Chinese hamster cells since many repair-deficient mutations would be expected to be incompatible with human life and are, therefore, only to be studied in culture. Chick cells have also proved useful for the study of repair of UV damage as they possess photoreactivating ability.

The identification and characterization of radiation-sensitive and other variants is not, however, an end in itself. Our studies have led to three distinct applications. Firstly, radiation-sensitive variants constitute the raw material for fundamental studies on human DNA repair and mutagenesis. We are currently shifting the emphasis of our programme away from the isolation of repair-deficient mutants (of which a large collection already exists) and towards (a) genetic and biochemical characterization of the radiation response of normal and sensitive cultured cells and (b) the elucidation of the pathways of DNA repair and mutagenesis, and their control using more sophisticated approaches (among them the use of recombinant DNA techniques).

A second important application of our work has been the revelation of the natural range of radiosensitivities among the human population at the cellular leve not merely the established sensitive variants, but also among the "normal" population, D_0 values for cultured fibroblasts from which extend from 97 to 180 rads. Suspicion is growing that some of this variability may be ascribed to individuals heterozygous for some of the major hereditary conditions thought to involve deficiencies in DNA repair, e.g. ataxia-telangiectasia. There is evidence that individuals heterozygous for this disorder may be abnormally cancer prone. We are currently placing more emphasis on the study of heterozygotes. In the long term the possibility exists that potential radiation workers might be screened to detect hypersensitivity to ionizing radiation but we shall require a much better understanding of the genes involved before such a screening approach could be contemplated with any confidence.

A third application of our work is in clinical medicine, in particular the development of methods for prenatal diagnosis. This is now possible for excision-defective XP individuals and as knowledge grows of the biochemical deficiencies it should be possible to extend the range of conditions for which prenatal diagnosis is possible. In addition, it is likely that our work will prove to have other clinical implications and not only in the conditions which we study.

The studies detailed in the following pages illustrate the various genetic and biochemical approaches which the collaborating laboratories have pursued as part of the integrated programme of research.

Contractor : Department of Cell Biology and Genetics
Erasmus University, Rotterdam, The Netherlands.

Contract nr : 196-76-1 BION

Head of the Research teams : Prof. D. BOOTSMA

General subject of contract : To identify and characterize various strains of mammalian cells deficient in repair of DNA damage.

Title of Project N° 1 : Isolation of radiation-sensitive mutants

Head of project and scientific staff: Prof.Dr. D. Bootsma and
Drs. Westerveld, Jaspers, Kleijer and Kei

Three different categories of cell lines have been isolated or collected in this project:

1. Primary fibroblasts derived from individuals suffering from genetic diseases in which defective DNA repair may play a role. Cell strains were obtained from about 120 different xeroderma pigmentosum patients. Other strains of interest originated from patients having ataxia telangiectasia, progeria, Werner syndrome, Bloom's syndrome, Fanconi's anemia etc.

In three cases of pregnancies at risk for xeroderma pigmentosum we performed prenatal diagnosis in cultured amniotic fluid cells (Halley et al. 1979).

2. Chinese hamster mutant cell lines sensitive to ultraviolet irradiation (UV) have been isolated from CHO cells by using a newly developped replica plating technique. The 3 lines that have been characterized showed decreased levels of unscheduled DNA synthesis, impaired postreplication repair and decreased colony forming ability after UV exposure.

3. Large panels of proliferating human-Chinese hamster and human-mouse hybrid cell lines have been isolated. These hybrids have been used for the localization of genes on human chromosomes for the characterization of the Philadelphia chromosome translocation in chronic myeloid leukemia and for the genetic analysis of DNA repair mechanism in human cells (see project 3).
We have investigated the induction of sister chromatid exchanges (SCE) by UV exposure of XP cells of different complementation groups (de Weerd-Kastelein et al., 1977). XP cells belonging to the groups A, B, C and D showed an increased sensitivity compared with normal cells. Group E cells did not differ from normal cells in that respect. In an XP variant cell strain it was found that SCE formation is increased after exposure of the cells during the S-phase of the cell cycle.

Title of project N° 2 : Biochemical characterization of radiosensitive mutants

Head of Project and scientific staff: Prof.Dr. D. Bootsma
Drs. N.G.J. Jaspers, J.J.H. Fortuin, W. Keijzer, J. de Wit.

1. Xeroderma pigmentosum.

Cell strains from patients presumed to have a genetic defect related to ultraviolet radiation hypersensitivity (project 1) were tested with respect to a number of biochemical parameters.

As mentioned in more detail in previous research reports a large number of XP cell strains was diagnosed by the unscheduled DNA synthesis test as being excision deficient.

In 24 cell strains we measured postreplication repair after UV exposure. Ten of these showed the pattern of excision-defective XP, among which were the strains XP23OS and XP2BI from the new complementation groups F and G, respectively; six strains had the XP-variant phenotype and eight were normal. In addition it was demonstated, that postreplication repair could be measured in human amniotic fluid cells as well, which made it possible to carry out prenatal diagnoses on three fetuses at risk of XP, using both UDS and post-replication repair tests.

Evidence was obtained for a sensitivity to ionizing radiation in a number of XP-variants. Two variants showed an increased amount of micronuclei induced by X-irradiation or tritium beta-rays. However, their cellular survival, and the repair of DNA breaks (both single and double stranded) were normal after X-ray exposure.

2. Ataxia telangiectasia

After exposure of ataxia telangiectasia (AT) cells to ionizing radiation or bleomycin the rate of semiconservative DNA replication was depressed to a significantly lesser extent than in normal cells. After treatment with a variety of other carcinogenic agents AT fibroblasts responded normally. These observations were made on all AT fibroblast strains tested, both excision repair deficient and proficient. The differences in DNA synthesis inhibition correlated with the cellular sensitivity to a particular agent. Similar effects could be seen in AT lymphocytes, which means that the DNA synthesis measurement can be used as a clinical test to support the diagnosis of AT. Lymphocytes from AT heterozygotes showed near-normal rates of DNA synthesis after exposure to bleomycine or X-rays. Using this test we identified a Dutch patient with clinical symptoms different from AT (79RD27). Its biochemical and cytogenetic characteristics were like those in AT patients.

Title of project N° 3 : Genetic studies on DNA repair mutants

Head of Project and scientific staff: Prof.Dr. D. Bootsma and
Drs. A. Westerveld, N.G.J. Jaspers,
S. Matsukuma, A. Geurts van Kessel,
B. Zelle, J.J.H. Fortuin, W. Keijzer
M. Stefanini, J. de Wit

The genetic studies include two main approaches:

(1) Genetic characterization of DNA repair mutants by complementation analysis and

(2) Localization on human chromosomes of genes involved in DNA repair.

1. Complementation analysis

1a Excision repair deficient XP

At the start of this project in 1976 we had identified 5 different complementation groups in xeroderma pigmentosum. A large number of new XP cell strains from all over the world was subjected to complementation analysis by cell fusion. This resulted in the detection of a new group, the G-group, represented by cell strain XP2BI (Keijzer et al., 1979) and XP3BR.

From 8 Egyptian XP strains 5 were assigned to group C and 3 to group A. (Hashem et al. 1980). These strains were of interest because of previously published evidence by El-Hefnawi et al. of a linkage between XP and the ABO blood group, based on studies of Egyptian families with XP. Russian XP strain XP4LE was assigned to complementation group C. The complementation pattern after exposure to UV was compared with that after treatment with 4NQO (in collaboration with Dr. Lohman and coworkers). A similar pattern of complementation was obtained following both treatments (Zelle et al., 1980).

The kinetics of complementation in heterokaryons after fusion of XP cells from different complementation groups was investigated. The defect of XP strains of the A group was complemented at a fast rate after fusion with normal or C-group cells. Slow complementation was found with regard to the C-group defect.

Complementation was also observed after fusion of XP cells with cytoplasts derived from normal cells or complementing XP cells. The occurrence of complementation in these socalled cybrids demonstrated the presence of

the repair proteins in the cytoplasts of cells. A similar kinetics of complementation as shown in heterokaryons was found in these cybrids: the A group factor being readily available and the C-group factor acting at a much slower rate.

A difference in complementation kinetics of A and C group cells was also observed after fusion with chick erythrocytes. In contrast with earlier published data by Darzynkiewicz et al. we found restoration of the repair activity in the XP nuclei during or after reactivation of the chick erythrocyte nucleus. In these heterokaryons complementation of the A group defect started within 2 days after fusion at a time that reactivation of the chick nucleus was not yet completed, whereas it took at least 4 days in the case of the C-group defect.

These different sets of data clearly demonstrated that A and C group XP cells are deficient in different gene products and that restoration of DNA repair after fusion of A with C group cells is due to intergenic complementation.

1b. Postreplication repair deficient XP

A procedure was worked out to measure postreplication repair after UV irradiation of fused cells. After fusion of XP variant cells with normal cells or with excision-repair deficient XP cells from group A, C, and D a normal pattern of postreplication repair was observed in the heterokaryons, indicating complementation. In contrast, no complementation was evident after fusion of six different XP variant strains from unrelated donors with varying clinical manifestations of the disease. This suggests, that the XP variants comprise a single genetic complementation group different from group A, C and D.

1c. Ataxia telangiectasia

The diminished inhibition of DNA synthesis after X-ray exposure is a biochemical abnormality common to all AT cells strains (see project 2). Based on this abnormality a procedure for complementation analysis of AT was developed, that could be used for all AT strains. The technique used, was a single-cell autoradiographic assay for the rate of DNA synthesis. In addition to the two complementation groups (A, B) found by Paterson et al. earlier, we identified two new groups sofar: C (AT4BI and AT262) and D (AT5BI). These observations indicate that also in AT there is extensive genetic heterogeneity comparable to XP.

2. Localization of human (repair) genes.

In this part of the project emphasis has mainly been on the development of techniques which could be used ultimately for the localization of genes involved in DNA repair. Several approaches have been followed:

a) the transfer of genes by means of isolated metaphase chromosomes and isolated DNA;
b) the characterization of human-human microcell hybrids;
c) the characterization of the repair capacity in proliferating human-rodent somatic hybrid cells;
d) the characterization of DNA-repair in cybrids obtained after fusion of XP cells with cytoplasts of human-Chinese hamster hybrid cells.

a). Transfer of the genes coding for hypoxanthine phosphoribosyl transferase (HPRT) and thymidine kinase (TK) was achieved by incubating HPRT or TK deficient rodent cells with chromosomes isolated from human HeLa cells (see Wullems et al., 1976 and 1977). After selection and isolation of the HPRT and TK positive clones it was shown that the selected rodent cell lines did express human HPRT or TK. In the experiments showing human HPRT transfer a few clones also expressed the X-linked phosphoglycerate kinase (PGK) gene. This cotransfer of genes was shown for TK as well. With the aid of specific chromosome staining techniques it was shown that some of the transfer clones possessed pieces of human chromosome material. Last year gene transfer could be obtained by introduction of isolated DNA fragments into mammalian acceptor cells. Using this DNA-transformation technique we were able to transfer human TK and HPRT to rodent acceptor cells. These transfer techniques can be used for each gene which can be selected for. We are applying these techniques in our experiments which aim the transformation of UV sensitive XP cells into UV resistant cells by incorporation of the wild type XP gene.

b). Characterization of microcell hybrids.

The microcells were isolated from HeLa cells or from a diploid human fibroblast cell strain. They were fused with diploid XP fibroblasts, HPRT deficient fibroblasts derived from a Lesch-Nyhan patient or HPRT deficient SV40 transformed XP cells. Restoration of the HPRT-activity and DNA-repair was observed in some of the hybrid cells as measured by ^{3}H-hypoxanthine incorporation and unscheduled DNA-synthesis following UV-irradiation.

Only the fusion of XP SV40 HPRT$^-$ XP cells with microcells derived from human diploid fibroblasts resulted in repair positive hybrid clones. However, the variability in the chromosome set of the SV40 transformed XP cell lines did not allow the identification of the chromosome(s) being introduced by the microcell. We concluded that this technique cannot be used for the assignment of repair genes on human chromosomes.

c). Characterization of proliferating human-rodent hybrids. Human chromosomes are preferentially lost in these hybrids and many homologous gene products of man and rodents can be distinguished. Correlation between loss or retention of human chromosomes and human gene products has resulted in the localization of a large number of genes on human chromosomes (see Westerveld er al., 1976 and other publications). We have tried to use a 50% difference in UDS level between human and Chinese hamster cells as a repair marker in hybrid cells. A number of hybrid cell lines isolated after fusion of normal human and Chinese hamster cells were characterized for their UV induced repair capacity. The data did reveal different repair values in hybrids with different human chromosome content. However, we were unable to correlate a specific level of UDS with the retention or loss of one specific human chromosome. This system will now be modified by using Chinese hamster repair deficient mutant cells in the fusions (see project 1).

d). During the last year of this contract we have developed a new procedure for mapping of one of the XP mutations on a human chromosome. This procedure is based on our studies of complementation kinetics in heterokaryons and cybrids. The fast rate of complementation of the A-group defect is used as a marker in cybrids obtained after fusion of UV exposed XP A group cells with cytoplasts of human-Chinese hamster hybrid cells. Our Chinese hamster parental cells were unable to achieve fast complementation of the A group defect. Therefore, restoration of DNA repair in the UV exposed XP nucleus in the cybrids is dependent on the presence of the human wild type gene product in the cytoplasts of the human-Chinese hamster hybrid. Correlation of presence or absence of human chromosomes in the hybrids with UDS levels found in the cybrids may reveal the human chromosome that carries the gene which is mutated in XP patients belonging to the A group.

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Contractor: State University of Leiden
Department of Radiation Genetics and Chemical Mutagenesis

Contract Number: 192-76-1 BIO N

Head of Research Team: **J.W.I.M. SIMONS**

General Subject: The genetic and biochemical basis of radiation sensitivity in human and other cells in culture

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Title of Project No. 1: Genetic analysis of DNA repair

Project Leader and Scientists: M. Hofker, Dr. A.T. Natarajan, Dr. J.W.I.M. Simons, Dr.Ir. A.A. van Zeeland

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1. Progress in the study of DNA repair processes is largely connected with the availability of repair deficient cells. Therefore, during the contract period, efforts have been directed at the isolation of repair deficient mutants in Chinese hamster cells. The first approach which has been followed was the isolation of 158 clones after a strong mutagenic treatment with EMS. From these clones 123 were tested for survival after a single dose of X-rays (800 k) or UV (10 J/m^2). An indication for radiation sensitivity was found in 27 clones, which have been retested with the determination of their survival curves after X-rays and UV. In this way a stable radiation sensitivity has been confirmed for five clones. Two of them (no. 1 and no. 145) were UV sensitive, one (no. 93) was X-ray sensitive and two (no. 24 and no. 36) were sensitive for both UV and X-ray. Because the evidence for a repair-deficiency in these clones appeared poor (see project 2 and 3) and because their radiation-sensitivity is only about 2-fold enhanced, experiments have been carried out to develop a method which would allow the isolation of rare radiation sensitive mutants. Excision repair deficient cells in G_1 will incorporate less BUdR after UV irradiation due to diminished repair replication. Because of this the cells are less vulnerable to the killing effects of UVA (long wave ultraviolet irradiation). Moreover this killing effect of UVA can be enhanced considerably by the addition of Hoechst stain. Reconstruction experiments are being carried out in which unirradiated cells represent repair deficient cells and irradiated cells as repair proficient cells. In this way it appears possible to sort out the optimal conditions for the selection of repair deficient cells. The

results so far obtained suggest that repair deficient cells can be 100 - 1000 fold enriched in a population by this procedure.

2. A patient diagnosed as Black fan Diamond (pure red cell aplasia) was examined for the response to UV mitomycin C and X-rays, as studied by increase in frequency of chromosomal aberrations, micronuclei and sister chromatid exchanges in the peripheral blood lymphocytes. The spontaneous frequencies of chromosomal aberrations in this patient is about 3 times higher than control. While the response to UV and MMC was normal in this patient, the frequencies of X-ray-induced aberrations and micronuclei were 2-fold higher.

3. The radiation sensitivity in human diploid skin fibroblasts was determined in nine wild type cell lines derived from normal individuals and in nine age-matched cell lines derived from patients with Huntington's Chorea. The D_o of the normal strains was 111.2 $\pm$ 13.3 with an extrapolation of 1.00 $\pm$ 0.14 while the D_o of the Huntington strains amounted to 113.2 $\pm$ 12.9 with an extrapolation number of 1.11 $\pm$ 0.25. These results show that the radiation sensitivity of cells from patients with Huntington's Chorea cannot be distinguished from the radiation sensitivity of normal cells. Moreover they do confirm that heterogeneity in the radiation response is present in the normal human population.

Title of Project No. 2: Biochemical Characterization of radio-sensitive mutants

Project Leader and Scientists: Dr.Ir. A.A. van Zeeland, Dr. J.W.I.M. Simons

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1. Analysis of excision repair of pyrimidine dimers in mammalian cells.

Quantification of pyrimidine dimers in DNA is important for the investigation of the repair mechanisms utilized by cells after irradiation with ultraviolet light. A widely used method for such purposes involves the chromatographic separation of acid digestion products of DNA containing radioactively labelled thymine residues. Because the technique relies upon resolution of very small amounts of dimerized bases from the unaffected bases, its sensitivity decreases at pyrimidine dimer frequencies lower than those produced by irradiation of about 10 J/m^2. However, the biological significant dose range for mammalian cells and repair deficient bacterial mutants lies below 10 J/m^2. A more sensitive assay has been developed in which UV specific endonucleases are used to introduce single strand scissions in DNA at the sites of pyrimidine dimers. The molecular weight reduction of such DNA, usually analyzed by velocity sedimentation on alkaline sucrose gradients, may be used to determine pyrimidine dimer frequencies. The sensitivity of this assay is limited by the difficulty of isolating DNA of high molecular weight. This difficulty has been circumvented in bacterial systems by introducing the endonuclease directly into cells permeabilized by detergent and then lysing the cells directly atop the alkaline sucrose gradients used for the analysis of molecular weight.

In this project we have developed an analogous technique for introducing endonuclease V from phage T4 injected E. coli directly into mammalian cells. This technique involves permeabilization of the cells by rapid freezing and thawing them twice, thereby rendering about 50% of the pyrimidine dimers accessible to T4 endo V. The percentage of pyrimidine dimers which are substrate for T4 endo V can be enhanced to nearly 100% by treatment of the permeabilized cells with a high concentration (2M) of NaCl prior to incubation with the enzyme preparation. Since the number average molecular weight of DNA from unirradiated cells is usually around 2×10^8 daltons, frequencies of pyrimidine dimers induced by very low doses of UV (less than 0.5 J/m^2) can be detected.

Loss of endonuclease sites from cellular DNA was observed during post-irradiation incubation of V79 Chinese hamster cells and several human cell strains. V79 Chinese hamster cells were able to remove about 25% of the UV-induced endonuclease sensitive sites from their DNA in 24 hours (5 J/m^2). This small but reproducible removal of dimers is in accordance with our measurements of the amount of repair replication in the same cells carried out with bromodeoxy uridine labelling and CsCl density centrifugation.

In contrast to Chinese hamster cells, normal human fibroblasts are able to excise pyrimidine dimers at a much higher rate (33% in 6 hr and 70% in 24 hr after 10 J/m^2). Under liquid holding conditions pyrimidine dimer frequencies were measured in normal human fibroblasts as well as in xeroderma pigmentosum cells (XP7BE, complementation group D). In the normal cells (10 J/m^2) 63% was excised in 1 day, 80% in 2 days and 91% in 7 days. In the XP-cells (1 J/m^2) 11% was excised in 1 day, 14% in 2 days and 31% in 7 days. Mutants No. 24 and 36 which were isolated from mutagenized populations of V79 Chinese hamster cells and showed enhanced sensitivity to UV-irradiation for cell survival, were analyzed for the amount of repair replication induced by 5 J/m^2. The extent of repair replication was not different from the amount detected in wild type V79 cells.

2. Analysis of post replication repair in mammalian cells.

Changes in size of nascent DNA strands after UV irradiation have been followed in order to analyze the capacity of mammalian cells to synthesize DNA on a damaged template. It has been shown that DNA synthesized at early times following UV-irradiation is relative small compared to DNA synthesized in control cells, whereas at late times cells are able to synthesize high molecular weight DNA even though the parental strand still contains UV-damage. This phenomenon is usually referred to as "post replication repair". We have studied the question whether pyrimidine dimers are blocking the elongation of daughter strand DNA and if so, how long these blocks persists, since eventually the cells are able to synthesize high molecular weight DNA. Addition of ^3H-thymidine to cells immediately following UV-irradiation will result in radioactively labelled daughter strand DNA that is covalently bound to unlabelled stretches of daughter strand DNA which were already present at the moment of UV-irradiation. These preexisting stretches of daughter strand DNA will therefore contain pyrimidine dimers. By permeabilizing the cells and adding T4 endo V we were able to cut off these dimers containing pieces of daughter strand DNA. The size of the remaining radioactively labelled daughter strand DNA was analyzed on alkaline sucrose

gradients and compared with the interdimer distance which was visualized by prelabelling parental DNA with ^{14}C-thymidine. The results show that in V79 Chinese hamster cells as well as in normal human fibroblasts daughter strand DNA synthesized during the first 15 minutes after UV-irradiation has a size equal or slightly larger than the interdimer distance. At later times this newly synthesized DNA is rapidly elongated up to 7 times the interdimer distance in 4 hours. Therefore pyrimidine dimers are only causing a block for the growth of daughter strand DNA during the first 15 minutes after UV irradiation.

3. Photoreactivation of pyrimidine dimers in Xenopus laevis cells in culture.

The capacity of Xenopus laevis cells to remove pyrimidine dimers by photoreactivation was analyzed using UV doses of 2-10 J/m^2 (254 nm). The cells were subsequently exposed to photoreactivating light (Phillips TL09 lamp, predominantly emitting 350 nm). 80-95% of the pyrimidine dimers were removed when the cells were exposed for one hour to this photoreactivating light. The effects of photoreactivation on biological endpoints such as survival and the induction of gene mutations was analyzed (see Project No. 3).

Title of Project No. 3: The consequences of DNA damage and repair

Project Leader and Scientists: Dr. P.P.W. van Buul, D.R. A.T. Natarajan, Dr. J.W.I.M. Simons, Dr.Ir. A.A. van Zeeland

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1. The radiation sensitive mutant isolated from V-79 Chinese hamster cells (see Project No. 1) were tested for the induction of mutations, chromosome aberrations and SCE's. Mutants No. 24 and 36, which are sensitive to both UV and X-ray showed no difference in mutation induction after X-rays compared with wild type while after UV-irradiation mutant No. 24 was slightly more mutable and mutant No. 36 appeared normal. Experiments with mutants No. 1 and 145, which are sensitive to UV indicated that mutant No. 145 was not different from normal in mutation induction after UV-irradiation whereas mutant No. 1 appeared slightly less mutable. Mutant No. 93, which is sensitive to X-ray appeared normal in mutation induction after X-rays. The induction of chromosome aberrations appeared strongly enhanced, compared to normal cells in mutants Nos. 24 and 145 after UV-irradiation and in mutants Nos. 36 and 93 after X-irradiation. Mutant No. 1 appeared normal after UV-irradiation in this respect. The spontaneous SCE frequency appeared only enhanced in mutant No. 24 whereas mutant No. 36 had elevated levels in some of the experiments. The data together provide no hard evidence for the presence of a repair deficiency in these mutants (see also project No. 2) but they do show that different cellular processes are involved in the production of mutations, chromosome aberrations and SCE's.

2. Four experiments were performed to study mutation induction after X-irradiation in air in human diploid skin fibroblasts derived from patients with Ataxia telangiectasia (AT). The cell line used was AT5Bi. Induction of mutation has not been observed. If the enhanced radiation sensitivity of the cells would correspond with an enhanced mutation induction, as appears

to be the case for cell lines from patients with classical Xeroderma pigmentosum the mutation induction would have been 3 times higher than in normal cells and such an enhanced mutation induction would not have been missed in these experiments. Therefore it is indicated that the induction of mutation after X-rays is not enhanced in AT cells. No information was obtained on the question whether AT cells are mutable by X-rays. Because of the extreme radiosensitivity of the AT cells the dose which could be applied was low. As under anoxic conditions much higher doses of ionizing radiation can be administered than under oxic conditions it was tested whether it was possible to obtain higher frequencies of induced mutation by high doses of X-ray in the presence of cysteamine and in nitrogen (experiments in cooperation with Dr. v.d. Schans, TNO, Rijswijk). The results indicated that in normal human skin fibroblasts much less mutants are induced per rad under anoxic conditions than after X-irradiation in air, which rendered this approach not suitable to study mutation induction in AT cells. When the cytotoxicity of AT and normal cells was determined after irradiation in air and after irradiation in nitrogen and in the presence of 20 mM cysteamine the ratio of the Do's from normal to AT cells changed from 3.1 when irradiated in air to 4.0 when irradiated in nitrogen in the presence of cysteamine. This means that the lesion(s) for which AT cells are sensitive became relatively more frequent under anoxic conditions. Under the anoxic conditions used the number of breaks per rad is about 7-fold reduced while the number of base-pair changes per rad remains unaltered. Therefore the data suggest that AT cells are defective both in the repair of base damage and of breaks. AT cells and normal cells do not appear to differ in their frequencies of spontaneous mutations.

3. When DNA-replication is inhibited in mutagenised cells excision repair can take place over an extended period, which modifies the initial DNA-damage and which will have consequences for the frequencies of biological endpoints. Such a liquid holding method has been developed for human diploid skin fibroblasts by keeping confluent cultures stationary over periods of 7 days or longer by means of conditioned medium. Under this condition recovery from radiation damage induced by ultraviolet light or X-rays is observed as an increase in cloning-efficiency. The repair-deficient human cell strains XP25Ro and XP7Be (Xeroderma pigmentosum from complementation groups A and D respectively) exhibit less but still discernable recovery after UV-irradiation and the same was observed for AT5Bi after X-irradiation.

Experiments on mutation induction indicated that the repair which takes place during liquid holding of UV-irradiated XP7Be cells practically abolishes the mutation induction while after liquid holding of wild type cells irradiated with UV (254 nm and 313 nm) still some induction of mutations is observed.

4. Photoreactivation of UV induced cell killing and mutations in Xenopus laevis fibroblasts.

Cells from Xenopus laevis in culture have the capacity to remove UV-induced pyrimidine dimers upon exposure with photoreactivating light (see Project No. 2). One hour exposure to fluorescent light (Phillips TL09 lamps with an emission from 305-450 nm and a maximum at 350 nm) causes a reduction of about 90% in the frequency of pyrimidine dimers. In parallel experiments, the extent of UV-induced cell killing detected by colony forming ability of the cells was strongly reduced. A technique to detect ouabain resistant mutants in these cell populations was developed. The frequency of mutants resistant to 10^{-6} M ouabain could be measured and UV-induced mutations to ouabain resistant were fully expressed after a post UV expression time of 2 days. Exposure of UV-irradiated cells for one hour to photoreactivating light reduced the mutant frequency with 96%. These results indicate a direct correlation between pyrimidine dimers and the induction of gene mutations as well as cell killing in this system.

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Contractor:	The Medical Biological Laboratory TNO, Rijswijk, The Netherlands.
Contract No.:	200-76-1 BIO N
Head of the Research teams:	Dr. P.H.M. Lohman
General subject of contract:	To identify and characterize variant strains of mammalian cells deficient in repair of DNA damage
Title of Project No. 1:	Isolation of radiation sensitive mutants
Head of project and scientific staff : Dr. P.H.M. Lohman	

During the contract period the collection of human and Chinese hamster cell lines mutated in different DNA repair mechanisms has been expanded.

Human cells.

In 1978 we have described that cells derived from patients with ataxia telangiectasia (AT) are sensitive to ionizing radiation. DNA damage in cells irradiated with ^{60}Co-γ-rays was studied in these cells and in normal human fibroblasts. The induction and removal of DNA lesions was followed by several techniques.

In the first place we used the in vitro enzymatic assay to monitor the presence of base-damage in the DNA of eukaryotic cells after γ-irradiation. In this method the lesions are recognized by specific endonucleases (present in an extract from Micrococcus luteus bacteria) which cause single-strand nicks close to the lesions. The number of nicks is then determined. We tried to improve the sensitivity of this method by attempts to increase the ratio between the base-damages caused by γ-rays and the directly induced DNA breaks, by varying the experimental conditions of irradiation. Irradiation under N_2 instead of O_2 had a favourable effect, since it hardly influenced the number of base damages per Gy, but led to a 2-fold decrease in the number of radiation induced breaks, resulting in a approximately 1:1 ratio for single-strand breaks and base-damages. In the second place, the effect of the presence of sensitizers (viz. TAN and PNAP) during the irradiation was studied. As expected, these compounds enhanced the sensitivity of the cells to γ-rays resulting in less survival per Gy. They also gave an increase in the number of base-damages, as judged by the γ-endonuclease assay, but the number of radiation induced breaks increased as well, so the 1:1 ratio under anoxia was maintained. The sensitivity of the method was considerably improved, however, when protective agents were used during irradiation. With

the protective agent cysteamine, present at 20mM during γ-irradiation, the absolute number of base damages per unit dose as scored by the in vitro enzymatic assay was not affected, whereas the number of single strand breaks in DNA was greatly reduced.

The improved assay was used to study normal cells and ataxia telangiectasia cells for their capability of removing base damages. We found that the presence of the protective agent during irradiation did not affect the subsequent repair of the base-damages, neither in normal nor in ataxia cells.

In the contract period we have extensively tried to find a possible difference between normal human cells and ataxia cells in the capacity of removing base damages induced by γ-rays. However, in contrast to the results described by Paterson et al. (Nature 260, 444, 1978), we never have found any significant difference in the response of both cell lines. This failure in detecting difference in repair capacity between ataxia cells and normal human cells also occurred when the same cell lines, identical methods and the same materials were tried as were used by the group of Paterson in Canada. Therefore, at the moment we believe that ataxia cells and normal human cells do not differ in their capacity to remove γ-ray induced base damages, as recognized by the in vitro enzymatic assay with the endonucleases of Micrococcus luteus.

As a second approach to investigate the repair of DNA damaged by ionizing radiation in ataxia cells we studied the amount of repair-replication as measured by the incorporation of (^{3}H)-thymidine in damaged DNA regions. In agreement with the previously described results of the group of Paterson, we found a decreased capacity of ataxia cells to perform repair replication in comparison to normal human cells, when both were irradiated with comparable doses of ionizing radiation. This reduction was independent of the difference in the duration of generation cycle between ataxia cells and normal human cells. These results indicate that the enhanced sensitivity of ataxia cells as measured by the survival of the cells may indeed be attributed to a DNA repair defect. With normal human cells γ-irradiation in the presence of cysteamine resulted in a decreased repair-replication, after irradiation. Most probably this is due to a protection against the induction of certain DNA damages that normally give rise to repair-replication. Also with AT-cells (AT3BI) a decreased repair-replication was observed after irradiation in the presence of cysteamine, in addition to the lower repair-replication that these cells already show due to the defect in their repair mechanism.

In a third approach we studied the repair of γ-irradiation induced DNA breaks in normal human and ataxia cells. Indications have been found sofar that the enhanced sensitivity of ataxia cells might be attributed to the fact that a small fraction of γ-rays induced DNA breaks is not repaired by these cells. In attempts to identify more specifically the repair defect in ataxia cells, we study the induction and repair of DNA breaks with different techniques, including a more sensitive one which allows the use of low irradiation doses. To this purpose three techniques have been introduced. Sedimentation in alkaline sucrose gradients after direct lysis of the cells on top of the gradients, and alkaline elution from membrane filters are used for the detection of single-strand breaks and possibly also cross-links, and for the detection of double-strand breaks the neutral elution method is applied.

When the repair of single-strand breaks induced by moderate to high doses (20-150 Gy) of γ-rays was studied, a rapid and slow repair could be distinguished, representing two different types of lesions. The induction of the rapidly repaired single-strand breaks could be prevented by the presence of cysteamine during irradiation. Both types of single-strand breaks were also observed in irradiated AT3BI cells; they are repaired at the same rates as in normal cells.

After low irradiation doses ($\leqslant$ 4 Gy), in normal cells more than 50% of the single-strand breaks were found to be repaired within 2 minutes after irradiation, whereas preliminary results with AT3BI cells indicate that here some 3-6 minutes are needed to reach this percentage.

With the sensitive neutral elution method double-strand DNA breaks appeared to be detectable at radiation doses (10-50 Gy) which are only slightly higher than biologically relevant doses for mammalian cells (the 37 % survival dose is of the order of magnitude of 2 Gy). Also for the γ-rays induced double-strand breaks we found a remarkably rapid repair of a large part of the lesions (50% is repaired within 10 minutes after irradiation), but repair does not continue at this high rate. A significant fraction of the breaks is repaired much more slowly ($t_{\frac{1}{2}}$ about 60 min). Our results indicate that in general 80-97% of the breaks has disappeared after 2 h of incubation. The repair of double-strand breaks in two strains of ataxia telangiectasia (AT3BI and AT2BE) did not differ significantly from that in normal cells.

When normal cells were irradiated in the absence of oxygen, a four times decreased induction of double-strand breaks was observed, whereas irradiation under nitrogen in the presence of cysteamine resulted in an eight-fold reduced induction of double-strand breaks. Repair of these breaks followed the same time-course as observed after irradiation in air.

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1. Induction of gamma-endonuclease sensitive sites by gamma-irradiation in mammalian cells and their cellular repair are not affected by the presence of thiol-compounds during irradiation. G.P. van der Schans and H.B. Centen. Int.J.Rad.Biol., 34, 550, 1978 (abstract).
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6. The induction and repair of double-strand DNA breaks in normal human and ataxia telang- iectasia cells exposed to ^{60}Co-γ-radiation, 4-nitroquinoline-1-oxide or bleomycin. G.P. van der Schans, H.B. Centen, P. H.M. Lohman. Proc. International workshop on ataxia telangiectasia, November 1980, University of Sussex, Brighton, United Kingdom, eds. B. Bridges and D.G. Harnden.

Title of Project No. 2: Biochemical characterization of radiosensitive mutants

Head of project and scientific staff : Dr. P.H.M. Lohman

In the past it has been found that DNA repair pathways in mammalian cells are of great complexity. Not only are the enzymatic steps very complex in nature, but also there exists a number of lesion-specific pathways. The objective of this study has been to identify lesions in DNA after either UV- or γ-irradiation and to follow the repair of these lesions in mammalian cells.

Ionizing radiation damage: DNA breaks versus DNA base damage.

As described under project 1 and 3, we studied the differences in repair of DNA breaks and DNA base-damage in human and Chinese hamster cell lines irradiated with γ-rays. This approach was shown to be considerably more successful when protective agents are used during the irradiation to modify the ratio between γ-rays induced DNA strand-breaks and DNA base-damage. The continuation of these experiments will hopefully lead to a better understanding of error-prone lesions in the DNA of mammalian cells treated with ionizing radiation.

Ultraviolet radiation (UV). a. Dimer- versus non-dimer lesions.

In this contractperiod we have completed our first study on the induction and repair of dimer- and non-dimer DNA lesions in Chinese Hamster Ovary (CHO) cells irradiated either with 254nm UV or with broad spectrum "near UV" (in the latter case two irradiation conditions were used, with peak wavelenghts of 290nm and 313nm respectively). The radiation sources used induce dimers and, depending on the wavelenght, a various amount of other, sofar unknown, lesions in the DNA of CHO cells. A comparative study was made of the induction of pyrimidine dimers, survival, the amount of excision repair, the induction of sister chromatid exchanges (SCE) and the induction of mutations leading to 6-thioguanine resistance.

With all three radiation sources it was found that in CHO cells the measured amount of DNA excision repair and the production of SCE's was directly correlated with the presence of dimers in the DNA. However, when survival and mutation induction were measured in the UV-irradiated cells, it was found that not only pyrimidine dimers, but also other, unknown, DNA lesions are of biological significance. These lesions are of particular importance for the "near UV" sources because of their relative abundance in relation to the dimers.

b. DNA repair and survival in UV-irradiated chicken embryo fibroblasts.

The role of the pyrimidine dimers in cell killing and DNA repair has been studied in 254nm UV irradiated chicken embryo fibroblasts. In contrast to human fibroblasts, these cells possess an active photo-reactiviting system (PR), i.e. a visible light requiring system for the monomerisation of pyrimidine dimers. By comparing UV-irradiated cells either illuminated or kept in the dark after irradiation, the importance of dimers could be studied.

Survival was increased when the cells were exposed to PR light. At the same time the number of induced dimers in the DNA of chicken cells decreased. In this way it could be demonstrated directly that dimers are primarily responsible for the deleterious effects of 254nm UV on the survival of embryonic chicken cells. The effect of PR on the amount of repair replication was less clear. PR had little effect on repair during the initial period, but in a prolonged experiment a strongly reduced repair replication was found. Probably dimers are responsible for the majority of repair synthesis, but in the initial phase the number of dimers left unrepaired by PR is sufficient for a normal rate of excision repair.

c. Repair of UV damage in xeroderma pigmentosum cells (XP).

The biochemical nature of the repair defect in XP was investigated by using a sensitive (enzymatic) assay for the detection of pyrimidine dimers, the predominant lesion induced in the DNA by 254nm UV. Seven XP-strains, chosen from the 7 complementation groups A through G, and one XP-variant were compared with normal human fibroblasts. The XP-variant showed a normal level of dimer removal, whereas 6 of the other XP-strains had a greatly reduced capacity to remove this DNA damage, in agreement with the level of unscheduled DNA synthesis (UDS) in these cells. The F group strain (XP230S), however, was found to remove more dimers from its DNA than was expected from the low level of UDS in this strain.

Both the F group strain (XP230S) and an E group strain (XP2RO) were studied in more detail. In XP230S, UDS was followed over a longer period than the two or three hours normally used in UDS analysis.It appeared that in these cells the rate of UDS remains constant, whereas in normal cells the initially high rate decreases after prolonged incubation to about the same level as the constant rate in XP230S. When compared over the same repair-period, dimer removal and UDS in XP230S showed approx. the same reduction in relation to these phenomena in normal human fibroblasts. In XP2RO it was shown that the reduced level of UDS is not caused by a shorter length of the repair regions in XP2RO DNA, but is solely due to reduction in the number of sites/unit of time removed by excision repair.

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7. The induction of gamma-endonuclease-susceptible sites by γ-rays in CHO cells and their cellular repair are not affected by the presence of thiol-compounds during irradiation. G.P. van der Schans, H.B. Centen, P. H.M. Lohman. Mutation Research 59, 119-122, 1979.

Title of Project No. 3: Genetic studies on DNA repair mutants

Head of project and scientific staff : Dr. P.H.M. Lohman

The influence of thiol-compounds on the induction of mutants in Chinese hamster cells by ionizing radiation.

Parallel to the studies with human cells described under project no. 1, the effect of ionizing radiation on Chinese hamster cells was investigated. Also with these cells the presence of protective agents during exposure to γ-rays results in a remarkable decrease in the number of directly induced single-strand breaks, whereas the number of certain DNA base damages is not significantly affected. With these cells, also the induction of mutations leading to 6-thioguanine resistance was measured. It was found that under the applied irradiation conditions (in a nitrogen atmosphere), at the same level of survival, the mutant frequency under the survivors is enhanced by the presence of the protective agent cysteamine during irradiation. It is concluded that probably base-damages that can be detected as γ-endonuclease susceptible sites are predominantly responsible for the induction of mutants in the Chinese hamster cells. Experiments are in progress to study whether also when irradiation is carried out in the presence of oxygen, DNA base damages play an important role in the induction of mutations.

Complementation analysis in xeroderma pigmentosum cells.

In cooperation with the other four contractors mentioned in the preamble of this report, we continued the characterization of, at present, 7 different complementation groups in XP.

The genetic basis of complementation in XP is still obscure. The 7 groups may represent mutations in 7 different genes involved in excision repair (intergenic complementation). However, the possibility of complementation between different mutations in the same gene (intragenic complementation) cannot be ruled out. In collaboration with the University of Rotterdam we have approached this problem indirectly by studying complementation in the case of repair of lesions produced by the carcinogen 4-nitroquinoline-1-oxide (4NQO), for which XP cells are also hypersensitive. The experiments revealed that the classification of the XP strains into complementation

groups with respect to 4NQO-induced repair coincides with the classification based on the repair of UV damage. In this respect the results obtained with 4NQO do not oppose the concept of intergenic complementation, but still the intragenic complementation cannot be excluded. Furthermore the results indicate that the gene products being defective in these XP complementation groups have a similar effect on the repair of different types of DNA lesions, revealing that these gene products operate in steps in the DNA repair process which do not show a lesion specificity.

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3. Influence of thiol-compounds on the induction in Chinese hamster cells of γ-endonuclease sensitive sites and of mutants by ionizing radiation. G.P. van der Schans, H.B. Centen, P.H.M. Lohman. Abstract of the meeting of contractants of the EEC. Biochemistry and Genetics of DNA repair. Gif-sur-Yvette, France, January 24-25, 1979.

Contractor:	MRC Cell Mutation Unit, University of Sussex, Brighton, United Kingdom
Contract No.:	166-76-1 BIO UK
Head of the Research teams:	Prof. B. A. Bridges, Sussex, United Kingdom
General subject of contract:	To identify and characterize variant strains of mammalian cells deficient in repair of DNA damage

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Title of Project No. 1: Isolation of radiation-sensitive mutants
Head of project and scientific staff: Dr. C. F. Arlett

On the basis of our original aims of establishing a collection of human cells which might prove defective in the repair of DNA damage, we now have available for study a bank of some 300 cell strains or lines. These are representative of normal individuals and a wide range of patients suffering from genetic diseases. They have been catalogued in the various research reports and represent our source material.

A major effort has been devoted towards establishing the limits of *in vitro* sensitivity for human cells towards a range of agents which damage DNA in various ways. Cellular sensitivity for ionising radiation had been studied in a variety of cell strains (Arlett and Harcourt, 1980). The overall range in observed D_0 values was 38–180 rads suggesting considerable variation in radiation sensitivity in human cells. The lower limit is represented by a set of 10 ataxia-telangiectasia (A-T) cell strains (mean D_0 57 $\pm$ 15 rads). We consider the mean D_0 value for normal cells to be 127 $\pm$ 17 (S.E.) rads. Intermediate sensitivity has been observed in cells from familial retinoblastoma patients, Fanconi's anaemia and Hutchinson-Gilford progeria. Since making these original observations we have found similar intermediate gamma-ray sensitivity in some but not all Huntington's disease cell strains (Arlett, 1980a), a single Friedreich's ataxia cell strain (Lewis *et al.*, 1979) and one (XP3BR) out of seven xeroderma pigmentosum (XP) cell strains (Arlett *et al.*, 1980b).

Cellular sensitivity to the lethal effects of UV light has also been studied in a number of cell strains (Lehmann *et al.*, 1977; Arlett, 1979, 1980b). All XP cell strains defective in excision repair show a considerable hypersensitivity to the lethal effects of UV when compared with cells from normal individuals. The excision competent but post-replication repair defective 'variant' XP cell strain show little of any enhanced sensitivity (Arlett, 1979, 1980b). Cell strains originating from Cockayne syndrome(CS) have also been confirmed in their UV sensitivity by us (Marshall *et al.* 1980; Arlett, 1979, 1980b). A unique cell strain with cellular sensitivity to UV (11961) has been isolated (Arlett *et al.*, 1978). A number of conditions with an undoubted actinic involvement such as Rothmund-Thompson syndrome, Dariers disease and Ferguson-Smith syndrome have also been investigated. No enhanced cellular sensitivity to UV light has been detected in fibroblast strains from patients suffering from these diseases.

Title of Project No. 2: Biochemical characterization of radio-sensitive mutants

Head of project and scientific staff: Dr. A. R. Lehmann

At the end of the last programme we had identified a biochemical defect in post-replication repair (now often called daughter-strand repair) in the variant form of xeroderma pigmentosum (XP). This provided the stimulus for a search for defects in DNA repair in cells derived from donors with other genetic disorders with possible indications of mutagen sensitivity, for example with multiple skin cancers or with light sensitive disorders. Most of these cell strains had normal responses at the cellular level. All XP variant strains had the pronounced defect in post-replication repair, and all the excision-defective XP's except those from complementation group E, had an intermediate defect (Lehmann *et al.* 1977). One cell strain, derived from a sun-sensitive child (11961), was hypersensitive to the killing action of UV. No defect was found in this strain in either excision or post-replication repair (Arlett *et al.* 1978). Similar properties were also found in other laboratories for cells from individuals with Cockayne syndrome (CS), another autosomal recessive sun-sensitive disorder. We instigated further studies on DNA synthesis in CS and 11961 cells. We showed that the overall rate of DNA synthesis was initially inhibited to the same extent in the normal and the defective cells, but in normal cells it recovered dramatically about five hours after UV-irradiation. This recovery was not seen either in 11961 or CS cells (Lehmann *et al.* 1979). The response in Cockayne heterozygotes was normal. The recovery in normal cells was not inhibited by fluorodeoxyuridine, an inhibitor of DNA synthesis, but it was prevented by cycloheximide, an inhibitor of protein synthesis. These results suggested that the recovery of DNA synthesis might be inducible (Lehmann *et al.* 1979).

We have also continued studies on the mechanism of post-replication repair in human cells. We investigated in detail the suggestion from the work of others, that recombinational exchanges might be associated with post-replication repair. We were able to reproduce the observations from other laboratories, that pyrimidine dimers were associated with daughter DNA strands, but extension of these studies suggested that this could well be an artefact caused by attachment of labelled DNA to pre-existing dimer containing strands rather than by exchanges (Lehmann,

Kirk-Bell and Arlett, 1977; Lehmann and Kirk-Bell, 1978). We also showed that the frequency of pyrimidine dimers in the region of the replication fork was similar to that in bulk DNA in normal cells, and that in XP variants the size of daughter-strand DNA after UV-irradiation corresponded accurately to the inter-dimer distance in parental strands (Lehmann, 1979).

The discovery of cellular hypersensitivity to ionizing radiation in ataxia-telangiectasia (A-T) (Taylor *et al.* 1975) stimulated a search for a biochemical defect in the repair of radiation-induced damage in these cells. No defect was found in the repair of single- (Taylor *et al.* 1975) or double-strand breaks (Lehmann and Stevens, 1977). An assessment of these and other data on A-T cells led to the hypothesis that the radiosensitivity in A-T cells could result from an inability to rejoin a very small fraction of the total strand-breaks, which had some special structure (Lehmann, 1977). This was consistent with the finding of hypersensitivity in A-T cells to bleomycin, an agent known to produce strand-breaks, but not altered bases in DNA. As with the radiation studies, however, no gross defect in the repair of strand breaks could be detected biochemically after treatment with bleomycin (Lehmann and Stevens, 1979).

Many of our routine assays for defects in excision repair were aided by the development of a rapid simple procedure for its measurement, and extension of these studies have provided a rapid complementation assay for XP cells (Lehmann and Stevens, 1980).

Title of Project No. 3: Genetic studies on DNA repair mutants
Head of project and scientific staff: Dr. C. F. Arlett

Three classes of experiments are employed in this project:(i) routine dose response survival curves which measure the lethal effects of a particular DNA damaging agent in a human cell strain. These experiments have allowed us to screen out mutagen sensitive cell strains from Project 1; (ii) mutation induction to 6-thioguanine resistance. Such experiments are demanding in both resources and time but do allow us to measure the consequences of any mutagen sensitivity or defect in DNA repair. Both excision repair-defective and post-replication repair-defective XP cell strains are hypermutable when treated with UV (Arlett, 1980b). XP3BR, a cell strain from the second representative patient from complementation group G (Keijzer *et al.*, 1979) is important in providing us with the first cell strain with any indications of a hypersensitivity to the mutagenic as well as the lethal effects of ionising radiation (Arlett *et al.*, 1980b). Indeed these cells differ markedly from A-T cell strains which appear to be hypomutable following treatment with ionising radiation (Arlett and Harcourt, 1978; Arlett, 1980b). The absence of hypermutability following treatment of A-T cells with ionising radiation may be contrasted with the hypermutability of XP cells on treatment with UV light (Arlett *et al.*, 1980b; Arlett, 1980b). This difference suggests that the enhanced tumour incidence observed in A-T patients is not related in an obvious way to the cellular sensitivity to ionising radiation and may, perhaps, be a consequence of defects in the immune system which are characteristic of these patients.
(iii) Cytological tests to measure sister chromatid exchanges (SCE). These experiments have been important in allowing us to distinguish cell strain 11961 from Cockayne syndrome. 11961 cells have a wild-type sensitivity to the induction of SCE whereas CS cells are hypersensitive to the induction of SCE by UV (Marshall *et al.*, 1980). These cytological observations on untransformed skin fibroblasts from CS contrast with results obtained with CS lymphoblastoid cell lines (Cheng *et al.*, Cancer Res., 38 (1978) 1601-1609). A similar discrepancy between the results obtained for transformed and untransformed XP fibroblasts in response to the induction of SCE by ethyl methanesulphonate has also been recorded (Heddle and Arlett, 1980). There are thus significant differences in the results obtained from the two cell types which suggests that transformed cells may have very different properties from untransformed cells and that caution must be exercised in the extrapolation of results obtained with such cells.

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Contractor: The University College of Swansea.

Contract No.: 163-761.BIO.UK.

Dr. James M. Parry.

Genetic change and DNA repair mechanisms in the yeast Saccharomyces cerevisiae (1976-1980).

During the course of the contract we have undertaken the development of genetic and technical systems capable of assaying in the yeast Saccharomyces cerevisiae the induction of DNA damage and genetic change after ionising radiation exposure.

Yeast strains were constructed capable of detecting the induction of recombination (both between and within genes), point mutation (both base substitution and frameshift) and chromosome aneuploidy after radiation exposure. Such strains were constructed in both repair proficient and repair deficient genetic backgrounds. Using such strains we are able to determine the role of the various repair enzymes of yeast upon the production of each genetic event.

Extensive studies have been performed of the induction of the above genetic events after irradiation of cells during specific stages of the mitotic and more recently the meiotic division cycle. The studies indicate that there are considerable differences in cellular sensitivity during the division cycle and the maximur induction of all the genetic events occurred in yeast cells irradiated in the G_1 phase of cell division. For future work under the contract we are now performing similar comparative studies in cells undergoing meiotic cell division. Such studies will enable us to determine the relative consequences of radiation induced DNA lesions during both somatic and reduction division.

Results of Project No. 1.

Head of Project and Scientific Staff: Dr. James M. Parry, Dr. Elizabeth M. Parry, Dr. David Sharp and Dr. R. S. Tippins.

Title of Project: The study of radiation induced chromosome aneuploidy in the yeast Saccharomyces cerevisiae.

The major aims of this project have been the development of strains of yeast capable of detecting at high efficiency the induction of chromosome aneuploidy in cultures undergoing both mitotic and meiotic cell division after radiation exposure. During the period of the project the major emphasis of our research effort has been placed upon the development of systems capable of detecting the products of abnormal chromosome segregations during mitotic cell division and the most important results of the project have been produced by our work in this area. However, 1980 has seen the completion of our effects to develop suitable meiotic assay systems which will in future provide the experimental basis for our study of the mechanism of radiation induced chromosome aneuploidy during meiotic cell division.

Mitotic chromosome aneuploidy in yeast may be assayed by the use of strain D6. This culture has been used to detect, using selective medium, the induction of monosomic colonies (2n-1) produced by chromosome loss during mitosis after radiation (both UV and ionising radiation) exposure. Although the experimental procedure routinely assays only the production of cells in which chromosome loss has occurred we have shown that on non-selective medium trisomic colonies (2n+1) are also detectable in half sectored colonies that result from chromosome non-disjunction (Parry et al 1979).

The results of our work with yeast strain D6 indicate that mitotic chromosome aneuploidy was induced by UV, X-rays and β-irradiation. The irradiation of stationary phase yeast cells with UV and X-rays results in the induction of mitoti aneuploidy only at doses which produced high levels of cell lethality. In contras β-irradiation (using tritiated water) results in the production of monosomic coloni at doses producing only low levels of cell lethality.

Variation in the culture age of radiation treated yeast strains (i.e. during the stationary at the exponential phase of growth) indicate that cells undergoing exponential growth show maximum sensitivity to radiation induced mitotic aneuploid When exponential phase cells were separated into discrete phases of the cell cycle on a zonal rotor we were able to demonstrate that the G_1 phase of cell division was the most sensitive stage to the induction of radiation induced chromosome aneuploidy.

The role of DNA damage and its repair in radiation induced mitotic aneuploidy has been shown by a number of experiments. Yeast cells exposed to radiation treatment and held under liquid holding conditions before plating and assayed for mitotic aneuploidy showed increases in cell viability and decreases in the frequency of monosomic colonies. After UV exposure, cells exposed to photoreactivating light treatment showed increased cell viability and reduced frequencies of monosomic colonies. Yeast strains defective in excision-repair were shown to be extremely sensitive to the induction of mitotic chromosome aneuploidy. The results obtained indicate that at least a fraction of the monosomic colonies produced after radiation exposure were the consequence of DNA damage and that this damage was subject to the action of cellular repair systems. However, it should be emphasized that a proportion of the yield of induced monosomic colonies were not sensitive to the action of DNA repair systems and the study of the mechanism of induction of such colonies will be of considerable interest.

Our studies upon radiation induced meiotic chromosome aneuploidy involve the assay of the production of disomic spores (n+1) produced by non-disjunction during the process of yeast sporulation under controlled conditions. Initial results have demonstrated that the maximum sensitivity of yeast cells to the induction of aneuploidy occurs when cells are irradiated immediately prior to metaphase. Comparative studies are also being performed of the rates of chromosome non-disjunction in cultures genetically marked for chromosome VII (developed by us) and for chromosome V (developed by Professor Magni). Such studies provide the experimental basis for the future analysis of the kinetics of radiation induced chromosome aneuploidy during both mitosis and meiosis.

Results of Project No: 2

Head of Project and Scientific Staff: Dr. R. S. Tippins, Miss T. North and Dr. James M. Parry.

Title of Project: An investigation into the role of the cell cycle in the response of yeast cells to the induction of genetic damage by ionising radiation.

The major aim of this project has been the development of experimental systems capable of assaying the effects and consequences of radiation damage during specific stages of the mitotic cell cycle of the budding yeast, Saccharomyces cerevisiae. Such systems provide the basis for the determination of the consequences of genetic damage during the cell cycle and the role of the various stages of the division cycle in the production of genetically modified cells. The necessary requirements of this project have been the development of systems capable of isolating sufficiently large numbers of cells at specific stages in the mitotic cell cycle and a series of yeast strains capable of assaying the induction of a wide range of genetic events.

To provide the cells necessary for the project we have made use of the technique of gradient separation on a zonal rotor. Exponential phase yeast cells have been separated on the rotor into cell populations made up predominantly of the discrete stages of the cell cycle, i.e. G_1, S and G_2, which may then be utilized in our radiation studies.

The literature contains many reports that describe stationary phase yeast cells as being in the G_1 stage of the cell cycle. However, our studies have demonstrated quite clearly that stationary phase cells and G_1 phase cells derived from the exponential phase of culture growth are significantly different in their radiobiological responses irrespective of their genetic background. We were able to demonstrate that G_1 cells were consistently more sensitive to the lethal effect of radiation exposure than stationary phase cells and that such stationary phase cells are more appropriately described as G_0 as they are outside the normal cell cycle.

As the zonal rotor separates cells on the basis of cell size an essential part of the calibration of the system has been the determination of the possible role of cell size upon the radiobiology of yeast cells. For this purpose we have separated yeast cells from both the exponential and stationary phase of cell growth on the basis of cell size (3.4 to 6.0 microns). In the case of cells derived from the stationary phase of the culture growth there was no evidence of any significant variation in the radiobiological effectiveness of radiation exposure across the range of cell size classes. In contrast, in the case of separated fractions of an exponential culture significant differences in sensitivity were observed which correspond to the proportions of cells present in each of the characteristic stages of the cell division cycle.

To assay the genetic effects of radiation exposure during the cell cycle we constructed a series of yeast strains capable of assaying the induction of the following parameters:-

a) the induction of mitotic crossing-over by the detection of homozygous products of recombination in heterozygous diploids.

b) the induction of mitotic gene conversion by the detection of prototrophs produced in heteroallelic auxotrophic diploids.

c) the induction of base substitution and frameshift mutation by the detection of prototrophs produced in auxotrophic haploid and diploid cultures.

d) the induction of mitotic aneuploidy by the detection of monosomic colonies in a culture carrying an extensively marked heterozygous chromosome pair. (described in detail in Project No. 1).

Strains were constructed carrying both repair proficient (RAD) alleles and repair deficient cultures carrying defective alleles of the genes regulating DNA repair in yeast (rad).

After treatment with both ionising and non-ionising radiations cyclic variations were observed in the induction of a number of genetic end points. In general, radiation exposure was found to produce maximum induction of mitotic recombination (both crossing-over and gene conversion) and mutation (both base substitution and frameshift) during the G_1 phase of the cell cycle when the cells were at their maximum sensitivity to the lethal effects of radiation. These observations were in contrast to those obtained with chemical mutagens which have their maximal effects upon S phase cells. The association observed between the maximum induction of lethality and the induction of the various genetic end points leads to the conclusion that similar DNA lesions lead to different genetic consequences during the cell cycle.

Of all the repair deficient yeast strains assayed for their cell cycle sensitivity a significant response was observed only in cultures carrying defective alleles of the RAD 50 gene. In such strains the resistance of S and G_2 was abolished. These results indicate an involvement of the gene products of the RAD 50 and the close association of sister chromatids in the repair of radiation induced DNA damage in yeast.

To provide further information on the nature of the cyclical changes in radiation sensitivity we have investigated the effects of a number of modifying treatments. Yeast cells incubated in the presence of the repair inhibitor caffeine after radiation exposure show reduced lives of cell viability and mitotic recombination during the cell cycle, particularly during the G_1 phase of maximum induction of both events. Similar experiments have also been performed in which irradiated cells were incubated in the presence of the protein synthesis inhibitor cycloheximide. Such incubation results in reductions in the lethal and genetic effects of radiation exposure. Such changes indicate that enzyme systems leading to a wide range of genetic events after radiation exposure are inducible and require the action of post-irradiation protein synthesis.

The work performed on this project over the past four years has provided us with a much improved understanding of the relationship between radiation exposure and genetic change during cell division in yeast. Further development of the project will involve the detailed study of nature and rates of repair of DNA lesions during the cell cycle. Such studies will complete the series of inter-related experiments and enable us to predict the potential genetic consequences of radiation induced DNA lesions in mitotically dividing cells.

Results of Project No: 3

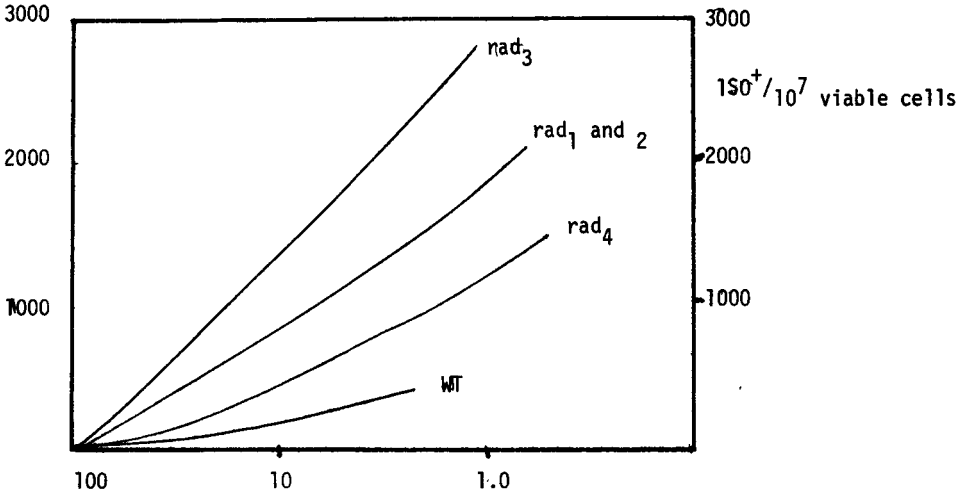
Head of Project and Scientific Staff: Mr. A. Hussain and Dr. James M. Parry.

Title of Project: The sensitivity of excision repair defective yeast cultures in ionising radiation.

The genes $\underline{RAD_1}$, $\underline{RAD_2}$, $\underline{RAD_3}$ and $\underline{RAD_4}$ of yeast have been shown to code for gene products involved in the excision repair pathway of UV induced pyrimidine dimers. Stationary phase cultures of strains carrying defective alleles of these genes show very large increases in cellular sensitivity to UV light exposure but are essentially wild type (RAD) in their response to X-irradiation. Wild type yeast cultures harvested during the exponential phase of growth show an increased resistance to X-ray induced cellular lethality compared to cells harvested from the stationary phase of growth. This exponential phase resistance of yeast cells to radiation exposure has been ascribed to the presence of budding cells. We have further investigated these "culture age" effects using a series of mutant cultures defective in each of the 4 genes involved in excision-repair described above. In all four mutant cultures called $\underline{rad_1}$, $\underline{rad_2}$, $\underline{rad_3}$ and $\underline{rad_4}$ the exponential phase resistance was not only abolished but the mutant cultures showed increased sensitivity to X-ray induced cell lethality compared to stationary phase cells and to wild type cultures.

We have further investigated the effects of X-irradiation in excision-deficient yeast strains by using assays of the induction of point mutation in a range of such strains. A series of assays have been performed of base substitution mutation at the isoleucine-1 locus. Typical data obtained from such experiments using exponential phase cultures are shown in Figure 1. The results demonstrate that the repair proficient RAD culture was relatively insensitive to the induction of base substitution mutation. In contrast, all four excision deficient strains showed increased sensitivity to the

Figure 1



induction of mutation. The results obtained indicate the involvement of the genes $\underline{RAD}_{1,2,3 \text{ and } 4}$ in the repair of potentially lethal X-ray induced lesions. The $\underline{RAD}_4$ gene was particularly interesting in this respect as strains carrying defective alleles of this gene were refractive to the induction of base substitution mutation after treatment with chemical mutagens.

Results of Project No. 4

Head of Project and Scientific Staff: Dr. James M. Parry, Dr. R. Waters, Dr. R. S. Tippins, Mr. C. Merrill and Mr. S. Kelly.

Title of Project: Studies upon the induction of DNA damage and genetic change during the meiotic cell cycle of yeast.

The aims of this project which was initiated in 1980 are the characterisation of ionising radiation damage produced by irradiation during the meiotic cell cycle and the determination of the genetic consequences of this damage. During 1980 we have undertaken a series of preliminary experiments designed to provide us with suitable genetic systems and experimental techniques capable of supplying us with information on the relative effects of radiation during both mitotic and meiotic cell division.

During the year we have undertaken calibration studies to develop suitable protocols for the extraction of high molecular weight DNA from yeast cells undergoing meiotic cell division. With the successful completion of these studies we utilized our protocols to determine the relative rates of DNA strand breakage and its repair across the cell cycle. Preliminary experiments have already indicated very considerable variation in the formation of single strand breaks and their repair, dependent upon the time of irradiation of a culture during meiosis.

To enable us to correlate the induction of DNA damage with genetic change in yeast we have undertaken a construction programme to produce strains capable of assaying the induction of point mutation (both base substitution and frameshift) and gene conversion during meiosis. As many of the yeast strains used for mutation experiments undergo meiotic cell division only at low levels it has been necessary to undertake considerable strain modifications to improve sporulation frequencies.

Results of Project No. 5

Head of Project and Scientific Staff: Dr. R. Waters and Miss J. Meredith.

Title of Project: DNA repair in irradiated human fibroblasts.

A number of projects are in progress concerning the replication and excision repair of DNA in irradiated human fibroblasts, and have been undertaken in collaboration with Drs. A. R. Lehmann and C. Arlett of the M.R.C. Mutation Research Unit, Sussex. The above processes have been studied in normal, various radiation sensitive homozygous cell lines, and in their heterozygous parents. Essentially three projects have been undertaken:

a) A study of the kinetics of excision repair and DNA replication in excision-deficient XP homozygotes and their heterozygous parents.

b) An investigation of DNA replication in normal and excision deficient XP cells receiving split doses of UV irradiation.

c) A study of the kinetics of DNA excision repair in both normal and UV sensitive Cockayne's syndrome fibroblasts.

Although still in progress, some general trends are apparent. To date:

i) No differences in the kinetics of excision repair (as measured with ara C), the extent of repair replication (^{3}H Brd Urd incorporation), or the amounts of DNA replicated after UV have been detected between normal and XP heterozygote cells.

ii) The application of split UV doses to excision deficient XP cells does not increase the total amounts of DNA replicated as seen with single doses. Sucrose sedimentation of pulse labelled DNA indicates that the first UV dose inhibits some replicon initiation that would occur if the second dose alone had been applied. Essentially, the data thus fit Painter's "model" for DNA replication events after split UV doses and do not support an inducible "by-pass" mechanism as proposed by D'Ambrosio and Setlow.

iii) Some changes in the kinetics of DNA repair have been observed in Cockayne's syndrome fibroblasts. However, these differences are not a characteristic of many of Cockayne's cell lines, and are under further investigation.

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Istituto di Genetica,dell'Università,Milan,Italy

Contract n. 153-76-1 BIO I

Head of the contract : Prof.G.E.Magni

MECHANISMS OF REPAIR AND MUTATIONS INDUCED BY X RADIATION IN SACCHAROMYCES CEREVISIAE

Project n. 1

Head of Project and scientif staff : G.E.Magni
L.Panzeri
S.Sora

Study of repair mechanisms on insertion/deletion and base substitution mutation induced by X-radiations.

The general aim of this investigation was to obtain evidence on the effect of repair mechanisms on the induction by X radiations of point mutations of known molecular nature, namely insertion/deletions (causing frameshift mutants) and base substitution(missense and non-sense mutations).

The approach consisted in determining preliminarly the precise molecular nature of point mutations to be investigated for the induced reversion. As a second step sensitive and resistant stage of yeast cell cycle were investigated and finally mutations affecting radiation sensitivity were introduced in our strains, the effect of X-radiations on the reversion of point mutations was analyzed.

1. The molecular nature of point mutations used for reversion studies

The exact knowledge of the molecular nature of point mutations used for reversion analysis and of their supressibility by external suppressor was an absolute prerequisite for the entire work. Particular attention was therefore dedicated to this subject as in Saccharomyces cerevisiae is often not easy to obtain clearcut evidence on the type of DNA change responsible for individual mutations.

To be utilized for our investigation each mutation had to satisfy to following requirements :

Non-sense mutations. It was considered sufficient their suppressibility by known non-sense suppressors. Such was the case of tyr7-1(UAA) and tyr7-1(UAG) completely suppressed by UAA and UAG suppressors respectively.

Missense mutations. The two mutants used ura4-10 and arg4-18 were classified according to the following criteria; ura4-10 was induced by 2-Aminopurine (proven by us to induce in yeast only AT -> GC transitions); arg4-18 is know to show non polarized complementation. Both mutants are not suppressible by non-sense

suppressors, revert at very high frequency to treatments with base substitution inducers (MNNG and HNO_2) and are practically insensitive to insertion/deletion inducers (ICR-170 and HYC). Insertion/deletion mutations; his4-519 was induced by ICR-170, hom3-10 arose spontaneously during meiosis; they are both suppressible by frameshift suppressors and unsuppressible by non-sense suppressors; they both revert efficiently to treatments with ICR-170 and Hycanthone and are almost insensitive to MNNG and HNO_2.

2. Effect of X-radiation on point mutations during the sensitive and resistant stage of cell cycle.

Yeast cells undergo a very significant variation of sensitivity to the killing action of X-radiation during their division cycle. From synchronized cultures it is possible to isolate by differential centrifugation cells showing maximal sensitivity (mean lethal dose 3.3-3.5 Krad, called from now on "sensitive") and a fraction at the maximal resistant stage (m.l.d. 35-45 Krad indicated as "resistent").

The mutagenesis data have clearly indicated that sensitive and resistant cells show no differences for anyone of the investigated genetic changes induced by X-radiation. The rates of induction are linear throughout a large range of doses and are reported in Table 1.

As far as mutations in non-sense triplets, both the rates of single base substitutions and of triplets changes are within the frequency estimated for E.coli.

For the reversion of missenses and frameshifts, the high reversion rates of the two frameshift mutations could be due either to a greater probability of induction of single-base insertions/deletions per rad or to a rather large number of mutable sites capable of restoring an acceptable frame. We prefer the latter hypothesis as it is highly probable that any base insertion or deletion could be compensated by other events in a rather large set of nearly bases.

If, for the sake of theoretical discussion, one assumes an equal sensitivity of all pre-mutational damage leading to base substitution, or base gain, or loss, our data could indicate that the two missense can be reverted by 1-3 base changes, whereas the frameshifts can be reverted by 10-20 base insertion or deletions.

Table 1

Rates of X-radiation induced mutations

Genetic event	Induced frequencies x 10^{11}
UAA $\rightleftharpoons$ UAG	0.2 - 0.4 events/base/rad
UAA, UAG $\longrightarrow$ sense	1.8 - 3.0 events/triplet/rad
ura3-10 $\longrightarrow$ URA^+	0.69 reversions/rad
arg4-18 $\longrightarrow$ ARG^+	0.44 reversions/rad
his4-519 $\longrightarrow$ HIS^+	2.10 reversions/rad
hom3-10 $\longrightarrow$ HOM^+	5.6 reversions/rad

3. Effect of rad mutations on reversions induced by X-radiation.

The aim of this part of our research was to investigate the effect of some rad mutations (affecting the survival of yeast cells to X-rays and/or U.V. light) on the reversion of frameshift and missense mutations induced by X-radiation. The following rad mutations were inrtoduced in our strains :

rad 1 : lack of excision repair

rad 18: belonging to the rad 6 group probably affecting an error prone repair

rad 50 and rad 51 : probably lack of single strand breaks repair

rad 52 : lack of double strand breaks repair

The effect of such rad mutations on the survival to X-rays is not reported, as our data are in agreement with those already well known.

Two of the rad mutation are endowed with a significant mutator effect as it shown in Table 2.

As far as the X-radiation induced mutations rad 1, rad 50, rad 51 and rad 52 have no effect on the induction of reversion in all four mutants analyzed. For rad 1 this was expected as it causes a lack of excision repair and it effects only U.V. induced mutation.

Table 2 Average of spontaneous reversion frequencies ($x10^9$)

Marker	wild type RAD^+	rad 51	rad 52
arg4-18	1.45	5.5	7.0
ura4-10	2.40	13.5	10.6
his4-519	1.80	30.5	25.3
hom3-10	33.0	56	89

N.B. The mutator effect of rad 51 and 52 seems to be rather aspecific as it is evident on both missense and frame-shift mutations.

The negative evidence on the rad 50 group indicates on the other hand that repair of single or double strand breaks is not involved in point mutations of whatever nature.
As shown in Table 3 the effect of rad 18 on X-radiation induced reversions is uniform in the sense of a reduction of mutation rates but with quantitative differences between missenses and frameshifts. The reduction of the reversion rate for mutants arg4-18 and ura 4-10 is in the order of 8-10 fold while that for his4-519 and hom3-10 is about 4 fold.
It is therefore confirmed that the gene RAD 18 is responsible for an error prone repair,possibly a little more efficient on base substitution mutations than on frameshifts.

Table 3 Induced reversion/rad (from 2.5 to 15 Kr) ($x10^{12}$)

Marker	RAD^+	rad 18
arg4-18	3	0.36
ura4-10	2.86	0.36
his4-519	10.6	2.6
hom3-10	30.0	7.6

We can conclude that the initial objectives of this project have been achieved,although the results did not turn out particularly exciting.X radiation is a very specific mutagenic agent as it can induce any type of mutational change in the gene either base

substitution of the transition and transversion type or base loss and gain with almost equal efficiency.
Repair mechanisms which are fully expressed in yeast "resistant" stage do not affect the rate of point mutation induced by X-radiation and this confirmes the hypothesis that such mechanisms repair lesion of the chromosome or chromatid breakage type. On the other hand most of genes involved in repair systems do not affect the action of X-radiation from either a qualitative or a quantitative point of view.
When they do so (rad 18) it seems that they affect base substitutions and base deletion/insertions more or less at the same rate.

Published papers :

Magni,G.E.,L.Panzeri and S.Sora
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Panzeri,L.,S.Sora and G.E.Magni
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Sensitivity to ionizing radiations and damage repair in yeast. Int.J.Rad.Onc. 5 : 1085-1088 (1979).

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Project n. 2

Head of Project and scientific staff : G.E.Magni
G.Lucchini
S.Sora

Study of chromosomal mutations induced by X-rays in Saccharomyces cerevisiae.

The aim of the proposed program was to investigate whether chromosomal deletions and translocations can be induced in yeast by X-radiation and to asses a fast procedure for their detection.

1. Chromosomal translocations

The possibility of making use of pseudolinkage during meiosis (the classical Drosophila procedure) was not taken into account due to the large number of chromosomes in Saccharomyces cerevisiae. An other typical character of heterozigous translocations is semi lethality of meiotic products. From direct tetrad analysis one would expect namely tetrads with spore survival of 4:0, 2:2,0:4 the first and last class beeing expected in almost equal amount, while 2:2 living/dead spores would be the result of X-over between the centromere and the translocation.

Some years ago we isolated a mutant which was widely investigated by Mac Kay and by us for the present project. The results of the two sets of experiments are given in the following table :

Cross	Spore survival in tetrads					
	4:0	3:1	2:2	1:3	0:4	
1375 (mutant) x wild type	23	3	54	5	15	(Mac Kay)
1375 x 10225 (wild type)	1	1	10	8	6	(our results)

These data indicate that translocations can be obtained in Saccharomyces cerevisiae but that the expected segregation for spore survival is strain dependent and therefore the analysis of this character must be restricted to a small number of presumptive translocations as a final check but cannot be used as first diagnostic parameter.

We then studied a selective procedure which was supposed to permit a selection of translocations between one arm of chromosome VII and any other chromosome. Out of 2750 clones analyzed in detail three turned out to satisfy the genetic requirements for a translocation. Further carefull analysis of the clones hetero-

zygous for a translocation,however, proved that they are too unstable. The great variability of genetic balance in cells of the same clone makes quantitative analysis too laborious. The line was therefore abandoned in 1978.

2. Chromosomal deletions

The system for detecting long chromosomal deletions was based on the pseudodominance effect on recessive genes carried in heterozygous state on chromosome VII. The detailed genetic analysis of more then 4000 clones after X-rays treatment proved that it waspossible to isolate few (three) aberrant clones carrying either point lethal mutations or very short deletions on chromosome VII. These three events were the final result of a series of rechecks on 132 presumed carriers of a heterozygous deletion.
This loss of aberrant clones is very relevant as it has been previously described and interpreted as due to mitotic rearrangement in strains carrying chromosomal aberrations and leading to the loss of the heterozygous aberration. Shounld such rearrangements have occurred in our material we lost most of the searched deletions,thus making a quantitative investigation very laborious and practically impossible.
We therefore conclude that our initial aim was not achieved. We got good evidence that translocations and deletions can be induced in yeast by X-radiation, but the general instability of cells unbalanced by chromosomal rearrangements makes any quantitative estimation tecnically impossible.
The project was consequently closed in 1979.

Contractant de la Commission : FONDATION CURIE
Institut du Radium
Paris

N° du contrat : 155-76-1 BIOF
Chef du (des) groupe(s) de recherche : R. LATARJET

Thème général du contrat : Lésions génétiques produites par les radiations et des polluants chimiques et leurs réparations. Comparaisons qualitatives et quantitatives en vue d'obtenir les "rads-équivalents".

Titre du projet N° 1

Repair of nuclear and mitochondrial genetic damages induced in yeast by radiations and chemicals.

Chef du projet et collaborateurs scientifiques : E. MOUSTACCHI, R. CHANET, F. FABRE, M. HEUDE, J.A.P. HENRIQUES, N. MAGANA-SCHWENCKE and C. CASSIER

The fate of yeast mitochondrial DNA (mitDNA) after irradiation.

The interactions between the nuclear and the mitochondrial genomes for the repair of radiation-induced lesions as well as the fate of mitDNA after several conditions of treatment were studied both genetically and biochemically. The situation can be summarized as follows :

1) The photoreactivation of mitochondrial UV-induced lesions occurs, and it is under the control of one nuclear gene PHR1.

2) The excision-repair process which is governed by 10 nuclear genes (RAD3 type) does not act on damaged mitDNA. The integrity of the mitochondrial genome is however required for an efficient repair by the excision process at the nuclear DNA level.

3) The fate of mitDNA after damaging treatments is strictly dependant upon the growth stage at the time of treatment :

a) when cells are treated in stationary phase the mitochondrial genetic markers are lost and a concomittant degradation of mitDNA is observed. It is concluded that mitDNA is not repaired in such conditions.

b) when cells are treated in exponential phase of growth a rescue of mitochondrial genetic markers is observed if an immediate plating is compared to delayed plating following a period of dark liquid holding. This is accompanied by a limited degradation, a reduction of mitDNA molecular weight and a restitution of the normal molecular weight. An accurate repair

system of the recombinational type appears to be involved in growing cells. This is under the control of several nuclear genes including the RAD6 type and at least three genes (uvsρ5 type) specifically involved in mitDNA repair. One mitochondrial determinant uvsρ72 is also concerned.

4) A genetic analysis of spontaneous and induced "petites" (rho^-) mutants shows that a portion of the mitochondrial genome is lost with a high probability. This preferential loss, related to the structure of the mitDNA (AT rich region) is likely to reflect a particular feature of this region in relation to replication as indicated by the restriction enzymes analysis of the mitDNA.

Recombination and repair.

The conditional cell division cycle yeast mutants cdc have been used to demonstrate that intragenic recombination induced by ultraviolet* or γ-rays occurs in diploids arrested in G1 (cdc4) a short time after irradiation and before the initiation of the S phase. This implies that pairing of homologous chromosomes does not require duplicated chromatids.

On the other hand, the cdc9 mutant is known to be defective, under restrictive conditions, in the rejoining in Okasaki fragments due to a ligase defect. X-ray induced recombinants within the cdc9 locus are produced with kinetics indicating that most if not all of the conversion events require the cdc9-controlled ligase. Thus the same DNA ligase is involved in DNA replication and in induced gene conversion.

Inducibility of a repair system in yeast.

1) Recombination between unirradiated chromosomes was induced by irradiation (UV and X-rays) of haploids followed by mating with heteroallelic diploids. The results show that recombination is repressed in mitotic cells and that one effect of radiation is to release a factor necessary for recombination. Lesions in the DNA do not participate to the recombinogenic events but are responsible for the induction of the recombinational ability.

2) A split-dose protocol shows that both UV mutability and resistance to killing are enhanced in cells liquid held after a "conditioning" dose of radiation ; these effects are blocked by the presence of cycloheximide during the liquid holding period. The magnitude of this enhancement in mutability is dependant upon the proportion of cells in S. Our results are consistent with the existence in yeast of major constitutive components of error-prone repair and a minor inducible component.

Radiation equivalence concept (REC), an example : formaldehyde.

The genotoxicity of formaldehyde (FA) as well as the major induced lesions in yeast DNA in comparison to γ-rays were determined in order to critically evaluate the REC notion. Some important differences in the response to FA and radiation are encountered depending upon the cells position in the cell cycle, the degree of ploidy and the repair ability of the strains. In general FA is a weaker mutagenic and recombinogenic agent than ionizing radiations.

The major lesions induced by FA in DNA include strand breaks and DNA-protein cross-links. Both types of lesions are repairable in wild type cells.

The establishment of meaningful rad-equivalent values requires appropriate knowledge relating to the differences in the mechanisms of action of the chemical mutagen and radiations.

Isolation and characterization of a novel class of mutants pso sensitive to photo-addition of psoralen derivatives.

1) We have shown that the three major pathways involved in the repair of radiation induced lesions are also required for the repair of psoralens photo-induced inter-strand DNA cross-links and monoadducts. A class of mutants specifically sensitive to psoralens plus 365 nm radiation has been isolated. Three of these pso mutants were analysed. They represent three new recessive genes as indicated by meiotic analysis and complementation tests with existing rad mutants. The defect in repair capacity concerns G1 and early S-phase cells. The pso2 mutant is also defective in G2 repair. The three mutants are mutationally defective and consequently govern an error-prone pathway. The interaction studies with the rad6 type mutants show that the pso genes define different steps.

2) The biochemical analysis of the induction of DNA cross-links and their repair in both nuclear and mitochondrial DNA was performed in wild type, rad3 (exc^-) and pso mutants.

The rad3 type mutant is defective in the incision of cross-links whereas the pso2 is blocked in the restitution of high molecular DNA which follows the cutting of DNA.

The major difference with the mechanism proposed for the repair of cross-links in E.coli resides in the fact that double-strand breaks are detected as an intermediary step in the repair of yeast DNA.

Titre du projet N° 2

Genetic effects and repair in Saccharomyces cerevisiae of furocoumarin plus 365 nm radiation (UVA) induced lesions and the rad-equivalent notion.

Chef du projet et collaborateurs scientifiques : D. AVERBECK and M. DARDALHON.

Furocoumarins are photosensitizing agents receiving much interest in fundamental and medical research work. The relatively high specificity of furocoumarin reactions towards DNA in the presence of 365 nm radiation (UVA) offers favorable circumstances for quantitative studies on the lesions produced in the genetic material and their repair. Furthermore, the world wide use of furocoumarins in cosmetics and in the photochemotherapy (PUVA) of certain skin diseases reinforces the interest in furocoumarin research work.

In this study special interest was focused on the genetic activity of mono- and bi-functional furocoumarins, the effects of dose-rate and the evaluation of rad-equivalents.

Genetic effects of mono- and bi-functional furocoumarins and UVA radiation and repair in the yeast Saccharomyces cerevisiae.

Two types of furocoumarins were used : mono-functional furocoumarins capable of inducing only mono-additions in DNA, and bi-functional furocoumarins capable of inducing mono- and bi-additions (interstrand cross-links) in DNA. In order to investigate the biological effect of the different types of lesions induced by furocoumarins, i.e., mono-additions and cross-links, we employed the bi-functional compounds psoralen and 8-methoxypsoralen (8-MOP) used in photochemotherapy and the mono-functional compounds 3-carbethoxypsoralen (3-CPs) (synthesized in our Institut by Dr. E. BISAGNI and co-workers), and angelicin. Our work focused on the genotoxicity of the compounds, and especially on that of 3-CPs which was shown to be also therapeutically active. Later on other mono- and bi-functional furocoumarins were included.

Survival data obtained in haploid and diploid yeast indicated that the cells were more resistant to the photo-addition of mono-functional furocoumarins than to that of bi-functional furocoumarins. A denumbering of the photo-induced lesions in DNA using a biochemical analysis showed that the higher resistance of cells to the induction of mono-additions was not due to differences in the number of lesions but mostly due to the differences

in the type of lesion induced. From this, it was concluded that the DNA cross-links induced by bi-functional furocoumarins such as 8-MOP are less efficiently repaired than the mono-additions in DNA produced by mono-functional furocoumarins such as 3-CPs and UVA*.

In this respect it was interesting that certain radiation-sensitive mutants showed different sensitivity patterns after treatments with mono- as compared to bi-functional furocoumarins indicating that some of the genetically controlled repair pathways for radiation-induced damage were operating in a differential way on the two types of photo-induced lesions. It was established that at least two different pathways, i.e., the excision-resynthesis pathway and a recombinational pathway contributed to the repair of furocoumarin plus UVA induced lesions. Very recently, this work was extended in Dr. Moustacchi's group and it appears that four pathways, the rad3 type excision resynthesis pathway, the rad6 type error-prone pathway, the rad50 type X-ray dependent pathway and a specific pso-gene dependent pathway are involved in this type of repair. The repair was shown to be dependent on growth phase.

In view of the different repair capacities for mono- and bi-additions the study of genetic effects of mono- and bi-functional furocoumarins was or particular interest. We measured the induction of nuclear genetic events in haploid and diploid yeast. Per unit dose of 365 nm radiation bi-functional furocoumarins were always more effective for the induction of reversions than the mono-functional furocoumarins. The same appeared to be true for the induction of forward mutations when for example, the action of 8-MOP (bi-functional) was compared to that of 3-CPs (mono-functional) in haploid yeast. These results hold also when expressed as a function of survival. These findings are in accord with the idea that in yeast the repair of lesions photo-induced by bi-functional furocoumarins is more error-prone than the photo-addition by mono-functional furocoumarins.

Per viable cell, the induction of mitotic crossing-over in diploid yeast was more efficient after treatments with bi-functional furocoumarins than after treatments with mono-functional furocoumarins. The induction of gene conversion was comparable in both cases.

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* The notion that furocoumarin induced bi-additions are less efficiently repaired than mono-additions was also verified with mammalian cell systems.

The potent mutagenic activity of bi-functional furocoumarins, such as 8-MOP, correlates well with their known carcinogenic activity in mice, whereas the reduced mutagenic activity of the mono-functional furocoumarin 3-CPs correlates well with its non carcinogenic effect in mice. Since this relation holds for the induction of mutations as well as for crossing-over (but not for gene conversion) carcinogenicity may be more related to the induction of mutations and intergenic mitotic recombination than to intra-genic mitotic recombination.

Mono- and bi-functional furocoumarins could be also distinguished by their different efficiency for the induction of cytoplasmic "petite" mutations (rho^-). At comparable survival levels mono-functional furocoumarins were always more efficient inducers of rho^- cells than bi-functional furocoumarins.

The differences observed in the genetic effects of the two types of furocoumarins permitted to characterize new photoreactive furocoumarins. The fact that the mono-functional furocoumarin 3-CPs was poorly mutagenic and non carcinogenic but therapeutically active on psoriatic lesions opened the way for the development of new psoralen derivatives which are less dangerous than the psoralens in actual use in PUVA-therapy.

Dose-rate effects observed with furocoumarins plus UVA in yeast.

Knowing the importance of dose-rate effects in radiobiology, it was interesting to investigate the effect of furocoumarins using low dose-rates of UVA radiation. When high (HDR) and low (LDR) dose-rates were compared, yeast cells appeared to be much more resistant to the action of 8-MOP (bi-functional) and 3-CPs (mono-functional) at LDR than at HDR. It was shown that the LDR-effect for 8-MOP is due to active repair processes involving to a large extent excision-resynthesis repair (rad3) but also other repair functions (rad6, rad9).

In the presence of 8-MOP the frequency of induced nuclear reversions and of mitotic crossing-over was lower at LDR than at HDR. In contrast, the induction of rho^- mutations at LDR was higher than at HDR for 8-MOP, but as high as that for 3-CPs at HDR when expressed as a function of survival. These findings suggest that in conditions of LDR 8-MOP induced damage in nuclear DNA is very efficiently repaired. This repair appears to be very limited in the case of 5-MOP which has a higher cross-linking

efficiency than 8-MOP.

Recently, also low dose-rate effects for gamma and UV (254 nm) radiation on yeast were demonstrated in our laboratory. The differences in repair kinetics observed make it appear worthwhile to pursue this program as proposed in the EURATOM project for 1981.

Evaluation of rad-equivalences for mono- and bi-functional furocoumarins.

Knowing that certain furocoumarins have been proven to be carcinogenic in mice and to enhance the risk for the induction of carcinoma in human patients treated with PUVA-therapy, it was thought to be useful to undertake a quantitative estimation of the genotoxic risk induced by different furocoumarins used in PUVA-therapy and in cosmetology. Since permissive doses and regulations do exist for ionizing radiation but not for mutagenic chemicals the task to establish rad-equivalences, i.e., a quantitative equivalence between a "dose" of a chemical mutagen and a dose of ionizing radiation producing the same effect in one or several biological systems of reference under well defined conditions, appeared to be of interest.

In yeast (Saccharomyces cerevisiae) we determined rad-equivalences for lethal effects, the induction of mutations and of intra- and intergenic recombination for four mono- and bi-functional furocoumarins, two of them (8-MOP and 3-CPs) of therapeutical interest. With the exception of intergenic recombination the rad-equivalences for genetic effects lay in the same range. Using data obtained on cultured mammalian cells a rad-equivalence could be also estimated for the phototoxic effect (cell killing) of 8-MOP in PUVA-therapy. The rad-equivalent value suggests that PUVA-therapy with 8-MOP should be handled with great caution with regard to long term genotoxic effects.

It is interesting to note that extrapolation of the rad-equivalence data to the genetic effects of PUVA-therapy gave about the same estimation of genetic risks as that already estimated on the basis of mutation studies in Chinese Hamster cells.

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Contractor :		National Radiological Protection Board, Harwell, Didcot, Oxon. OX11 ORQ England
Contract No.	:	171-76-1 BIO UK
Heads of Research Team	:	Dr. J. A. Dennis and Dr. H. Smith
General subject of contract :		Radiation-induced chromosome aberrations

Title of Project No. 1	:	The effect of changes in temperature and oxygen tension on the yield of radiation-induced chromosome aberrations in human peripheral blood lymphocytes
Head of project and scientific staff :		Dr. D. C. Lloyd, Dr. R. J. Purrott, J. S. Prosser

Variations in dose rate, dose fractionation and radiation quality are known to influence the shape of radiation-induced cell survival curves. Corresponding changes in the shape of dose response curves for cytogenetic damage induced by radiation to cells in vitro can be obtained. Such observations have contributed to the hypothesis that much of the cell killing in dividing cell systems can be related to the induction of gross chromosome anomalies which lead to genetic imbalance. It has thus become apparent that chromosome studies play a useful role in understanding several radiobiological phenomena of interest to radiology and radiological protection.

Much data exist on the effect of oxygen concentration on radio-sensitivity as measured by cell survival. Early experiments with Tradescantia indicated that oxygen enhancement ratios (OER) could be produced for cytogenetic damage but there are few reliable data on this derived from human or indeed from animal cells. This project was undertaken to provide experimental data on the effects of radiation on the chromosomes of normal human cells under conditions of different oxygen tension.

An apparatus was devised which permitted the oxygen concentration of heparinised samples of human blood to be varied and monitored over the complete range from anoxic (2 ppm) to fully oxygenated. The blood was maintained throughout at a constant temperature and then irradiated.

Dose response curves for dicentric aberrations with 250 kVp X-rays were produced under the two extreme conditions of equilibration with oxygen-free-nitrogen and with pure oxygen[1,2]. The data were fitted to the linear quadratic model. Measurements of the OER from these curves ranged from 7.2 at zero dose to an almost constant level of about 3 at doses above about 1 gray. This variation is probably due to a differential effect of oxygen concentration on the α and the β yield coefficients. Although the constant dose modifying factor theory of oxygen action cannot be rejected on statistical grounds, application of the linear quadratic model to the dicentric data favours a constant effect modifying factor hypothesis. In the presence of oxygen the dicentric yield induced by a given X-ray dose is increased at all levels of damage by a constant factor of about 7.

Variation in dicentric yields by a given dose of radiation was examined at different oxygen tensions[1]. This showed that chromosome damage is particularly dependent on oxygen concentration over a limited range (10^2 - 10^4 ppm). At both high and very low concentrations the dicentric yields tend towards constant values.

Linear quadratic dose response curves were also obtained for aberration induction by 14 MeV neutrons. The OER of 3.7 at zero dose and 1.6 at high dose was shown to deviate statistically from a constant indicating that oxygen was not acting as a dose modifying agent. RBE values obtained by comparing these neutron data with the 250 kVp X-ray results were larger at low neutron dose where the presence of oxygen did not influence the value obtained. At high doses the RBE was greater for anoxic irradiation, probably as a result of the greater contribution of single track damage induction by neutrons than by X-rays under these conditions.

The research was extended to consider the effect of adding to the system 2 hypoxic cell radiosensitising drugs, Metroindazole and Misonidazole, both electron affinic oxidising agents. The latter has shown more promise in clinical trials. Both drugs significantly raised the yield of induced aberrations, although had no enhancing effect on the yield in blood at normal venous oxygen tension. Over the dose range studied (0.25 - 5 gray X-rays) enhancement ratios ranged from 2 to 1.5 for Metronidazole and 2.8 to 2.2 for Misonidazole[3,4]. Similar values have been obtained with other biological systems *in vivo* and *in vitro* and therefore with the cytogenetic end point Misonidazole also appears to be the more effective drug. The effect of these

radiosensitisers on neutron induction of chromosome damage was also examined. Initial results suggest that any enhancement of dicentric induction will be small as expected for radiations having a low value of OER.

An experiment was carried out on the effect of simultaneous X-radiation and hyperthermia (39 and 41°C). Neither temperature showed a significant effect on the induction of aberrations from that found at 37°C. Oxygenated and anoxic blood held at 20, 37 or 41°C was also exposed to X-rays and examined for chromosome aberrations. There is some evidence from cell survival assays to suggest that hyperthermia may have a greater radiosensitising effect on anoxic blood. This was not confirmed by our results which showed the same aberration yield at each temperature. It is intended to repeat efforts to culture lymphocytes irradiated at temperatures greater than 41°C.

The examination of the effect of oxygen and the two radiosensitisers on the chromosomes of irradiated lymphocytes has provided enhancement ratios for normal human cells in agreement with values derived from animal experiments. In addition, because of the ability of the cytogenetic technique to provide results at doses below 1 gray, the applicability of the dose modifying theory of oxygen was shown to be limited. This theory cannot satisfactorily explain results over the entire dose range if aberration yields are fitted to the linear quadratic model, and this was particularly illustrated by results from neutron irradiation. Metronidazole and Misonidazole were found to sensitise only anoxic lymphocytes to X-rays with Misonidazole showing the greater effect.

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Title of Project No. 2 : The dependence of radiation-induced chromosome aberrations on radiation quality

Head of project and scientific staff : A. A. Edwards, Dr. D. C. Lloyd, Dr. R. J. Purrott, J. S. Prosser

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The objectives of this study were to obtain data on the yield of radiation-induced aberrations in human lymphocytes as a function of radiation dose and quality; and to use these data to test models of biological effects. On the basis of previous studies[1,2], the dicentric yield (Y) for X, γ and neutrons was found to vary as a function of dose (D) as:-

$$Y = \alpha D + \beta D^2$$

α and β being coefficients.

This equation is consistent with several microdosimetric models, notably those of Neary and also of Kellerer and Rossi, which attempt to explain the formation of aberrations. Alternatively, the concept that the ability of lesions to repair is dependent on the dose received by a cell also leads to models which approximate to the equation.

For fission spectra a good fit results when the β coefficient is zero. For 14 MeV neutrons, 7.6 MeV neutrons generated by the bombardment of 16 MeV deuterons on beryllium and the low LET radiations, β was constant within the experimental error. However, the α coefficient increased as neutron energy decreased and, in fact, there appeared to be a linear relationship with track average LET.

The more recent work with 14 MeV neutrons agreed well with previous measurements. The yield using californium-252 fission spectrum was linear with dose and the value of the α coefficient fitted well the relationship between α and track average LET previously derived empirically[3]. Results for irradiation with 30, 60, 120 and 150 KeV X-rays were obtained. The dicentric yields were similar to those obtained with 250 kVp X-rays of mean energy about 100 KeV. Analysis in terms of the equation failed to show any marked systematic variation of the α coefficient with X-ray energy.

A major contribution to this project has been the irradiation of blood with α particles[4,5,6]. A thin (32 μm) layer of blood was irradiated at normal incidence by 4.9 MeV α particles derived from ^{242}Cm. In another experiment ^{239}Pu citrate was added to blood for 24 hours and removed by centrifugation. Both experiments resulted in a linear dose effect relationship but with the α coefficient about a factor of three lower than that observed after exposure to fission neutrons. The RBE, for example, for 4.9 MeV α particles with respect to 250 kVp X-rays at low doses was 6. A model predicting this lower than expected value of RBE was proposed. It assumes that there is a selective removal of damaged cells by interphase death or as a result of mitotic delay, leading to an underestimate of dicentric yield. This model was tested by a method which measures these two phenomena and it was shown that they depress the RBE value by approximately a factor of three.

The distribution of aberrations in cells irradiated with various qualities of radiation was also examined[7]. For X-rays, γ-rays and fission spectra neutrons the distribution of dicentrics accords with Poisson statistics but for higher energy neutrons and α particles, over-dispersion was observed. The possibility that over-dispersion results from the variations of dose in sensitive sites leads to the conclusion that for dicentric formation the site size is about 7 μm diameter, that is close to the size of the cell nucleus.

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Contractor: United Kingdom Atomic Energy Authority
Atomic Energy Research Establishment
Harwell, Oxfordshire, OX11 ORA, U.K.

Contract nr: 167-76-1 BIO UK
Head of the research team(s): Dr D H Peirson

General subject of contracts: RADIATION SPECTROMETRY AND MICRODOSIMETRIC STUDIES

Title of project nr 1: Measurement of Neutron Absorbed Dose with Ionisation Chambers

Head of Project and scientific staff: H J Delafield, K G Harrison, P D Holt, J A B Gibson, S J Boot

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1. Introduction

This work was undertaken to improve the measurement of neutron dose in radiobiological studies, which were aimed at providing underlying data relevant to the establishment of radiological protection norms. Paired ionisation chambers have been widely used for the dosimetry of the mixed neutron and gamma-ray fields. A major problem in their use is the lack of information on the variation of the mean energy loss per ion pair (W) in the chamber gas with the type and energy of the secondary particles produced by neutrons. In order to investigate this problem, measurements of the specific ionisation in acetylene and carbon dioxide, using ionisation chambers of CH equivalent plastic and graphite, and monoenergetic neutron beams, have been made and compared with calculation. The need for this work became more apparent from an analysis of the Harwell measurements made at the ENDIP international intercomparison (Delafield, Boot and Holt, 1976), which showed that the technique was limited by uncertainties in the W and kerma values. This project was terminated in December 1977, and therefore this final report covers the years 1976-7.

2. Technique

A hydrogeneous chamber is used to measure the total dose, together with a neutron insensitive chamber for measuring the gamma-ray dose. The neutron dose, D_m, to the wall of the ionisation chamber is derived from the measured ionisation per unit mass of gas, J, by the Bragg-Gray theorem,

$$D_m = S_{m,g}\ \bar{W}_n J/e$$

Where $\bar{W}_n$ is the mean energy loss per ion pair in the gas, $S_{m,g}$ is the mean stopping power ratio for the wall and the gas and e is the electronic charge (ICRU). The neutron dose in tissue D_t is then derived from D_m using a theoretical kerma ratio K_t/K_m calculated for the neutron spectrum. For a homogeneous chamber $S_{m,g}$ is usually assumed to be unity, and so the accuracy of the technique is dependent upon a knowledge of the appropriate values of $\bar{W}_n$ and K_t/K_m.

Extensive calculations of $\bar{W}_n$ values were performed by Dennis (1973),and Dennis and Edwards (1975), who used an empirical model for the variation of W(E) with ion energy.

The overall uncertainty of calculated kerma factors has been steadily decreasing (ICRU 1977). For hydrogeneous materials at neutron energies below 2 MeV, the uncertainty in the kerma ratio K_t/K_m is small (<3%) whilst that in $\bar{W}_n$ is larger (8%). At higher neutron energies (∿15 MeV) kerma values are less well known, reflecting the uncertainty in the cross-sections for the inelastic and non-elastic reactions in carbon and oxygen (Dennis 1973). However relative measurements by Delafield, Chuang and Holt (1975)* of the ionisation produced by 15 MeV neutrons in various gases (acetylene, ethylene, T.E. gas, carbon dioxide and ethylene/carbon dioxide) commonly used in neutron dosimetry showed good agreement (<5%) with the calculations of Dennis. This provides confidence in both the relative cross-sections and W values used in the calculations at 15 MeV.

Measurements of the specific ionisation in acetylene and carbon dioxide were therefore made at neutron energies below 2 MeV where the value of W is least well known. The overall objective was to compare these measured values of ionisation with calculated values of Dennis (1973) and Dennis and Edwards (1975).

3. Ionisation measurements in acetylene and carbon dioxide irradiated with neutrons

Ionisation measurments were made with a homogeneous chamber having walls of a CH-plastic and an acetylene filling, and with a second chamber having a graphite wall and carbon dioxide filling (Delafield and Harrison, 1979). Four neutron energies were used between 0.24 and 1.7 MeV, the neutron fluences were measured with a de Pangher Long Counter which had been previously calibrated at the UK National Physical Laboratory and the spectra were checked by a time-of-flight technique. The measurments were corrected for gamma-ray contamination of the neutron beam with an energy-compensated GM counter.

The measurements are compared with the calculations based on the W models of Dennis (1973) and Dennis and Edwards (1975) in Fig 1. For acetylene the good agreement shown in the figure between experiment and theory is consistent with both W models and an assumed stopping power ratio of unity. The revised model of Dennis and Edwards (1975) is however to be preferred as it gives a better fit to the most recent experimental data on W for protons. A revised W model employing three parameters to obtain a better fit to the data was constructed by us from the most recent experimental data on W. However, this model did not give a significant improvement in the agreement between experimental and theoretical values of ionisation in acetylene over that obtained (±8%) by the later W model of Dennis and Edwards (1975).

Larger discrepancies (≲25%) were observed between the calculated and measured ionisation in carbon dioxide (Fig 1). This is thought to reflect both larger uncertainties in the measurements and, in particular, larger uncertainties in the calculation of kerma resulting from significant resonances in the oxygen cross section for three of the neutron spectra used.

4. Derived $\bar{W}_n$ values

'Experimental' values of $\bar{W}_n$ have been derived from the quotient of the calculated kerma (Dennis 1973) and the measured specific ionisation. In Table 1 these experimental values are compared with calculated values after

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Table 1 Comparison of derived and theoretical values of $\bar{W}_n/W_\beta$

Mean neutron energy (MeV)	Acetylene $\bar{W}_n/W_\beta$ [1]		Carbon dioxide $\bar{W}_n/W_\beta$ [1]	
	Experiment	Dennis and Edwards (1975	Experiment	Dennis and Edwards (1975)
0.236	1.14±0.07	1.15	1.57±0.20	1.72
0.554	1.22±0.07	1.13	1.69±0.17	1.53
1.12	1.13±0.08	1.11	1.65±0.20	1.36
1.72	1.07±0.09	1.10	1.42±0.18	1.30

1. Date normalised to W_β = 25.7 eV/ip for acetylene

W_β = 32.9 eV/ip for carbon dioxide

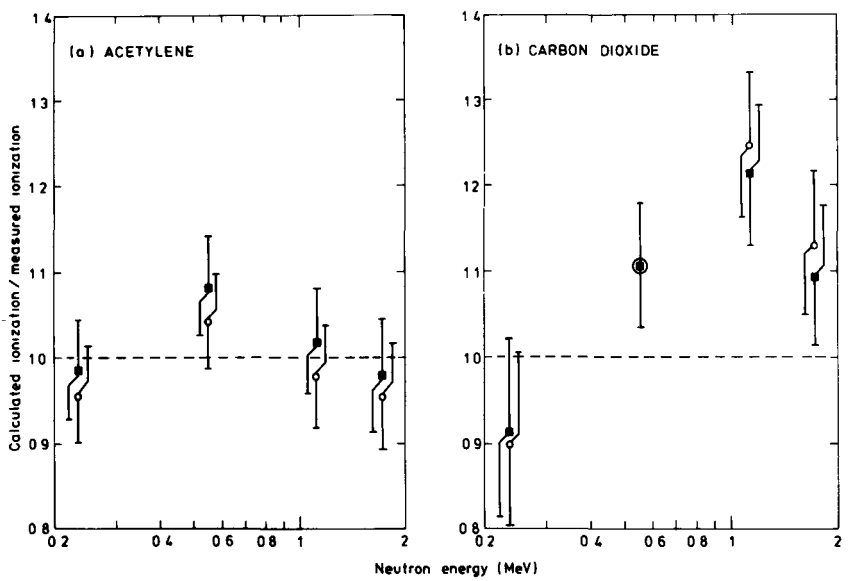

FIG 1 RATIO OF CALCULATED TO MEASURED VALUES OF SPECIFIC IONIZATION IN ACETYLENE AND CARBON DIOXDE o, CALCULATION BY PROGRAM OF DENNIS (1973), ■, CALCULATION BY PROGRAM OF DENNIS AND EDWARDS (1975), WITH ERROR BARS REPRESENTING UNCERTAINTY IN MEASURED IONIZATION ONLY

normalisation to the W value for electrons (W_β). The experimental results are consistent with an increase in $\bar{W}_n$ with decreasing neutron energy as expected from theory.

5. Conclusion

The direct comparison between the experimental and calculated values of the specific ionisation in acetylene and carbon dioxide irradiated with neutrons has provided both general confirmation of the theory (Dennis 1973, and Dennis and Edwards 1975) from the good agreement observed in acetylene, and shown up the limitations of the theory when applied to carbon dioxide.

For the neutron energy range studied (0.2-2 MeV) the observed agreement of better than ±8% between the calculated (Dennis and Edwards, 1975) and measured values of ionisation in acetylene gives confidence in using the CH chamber for neutron dosimetry, since the additional uncertainty in deriving kerma in tissue from that measured in CH-plastic is very small (2-3%). A representative value of $\bar{W}_n/W_\beta$ in acetylene for this energy range may be given by the mean of the experimental observations as (1.14±0.03) since the ratio is not markedly energy dependent (Table 1).

In contrast the neutron sensitivity of the graphite chamber filled with carbon dioxide is not so well known, and its use to measure the gamma-ray contamination of neutron beams cannot be recommended. The mean value for $\bar{W}_n/W_\beta$ of (1.58±0.06) derived from the measurements in carbon dioxide (Table 1) provides direct experimental evidence that $\bar{W}_n$ is significantly greater than W_β as predicted from the calculations of Dennis and Edwards (1975).

For gamma-ray measurements it is recommended that further studies should be made of other detectors with a much lower neutron sensitivity, such as an energy-compensated GM-counter, thermoluminescent dosimeters and photographic film.

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Title of project nr 2: Neutron and LET Spectrometry in a Man-Phantom

Head of Project and scientific staff: K G Harrison, Mrs A J Taylor, P D Holt, S J Boot

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1. Introduction

The aim of this project was to improve our knowledge of the neutron spectrum, dose and dose-equivalent in a homogeneous phantom, and to check by an independent method the depth-dose and depth-dose equivalent Monte Carlo calculations of Auxier, Snyder and Jones (1,2), which form the basis of the fluence to dose-equivalent conversion factors recommended by the ICRP (3). Techniques for both calculating and measuring neutron spectra in a phantom were therefore developed and used. An existing computer program which calculates dose and dose-equivalent from the neutron spectrum (4) was also tested by comparing the event-size spectra it predicts with those measured experimentally using a Rossi counter.

Following the publication of a further ICRP report (5) in 1977 (which introduced a concept in which the dose-equivalent imparted to individual organs of the body are weighted by risk factors and summed to give the total (effective) dose-equivalent), it is apparent that future work ought to be undertaken on realistically-shaped heterogeneous phantoms. This has been considered in the conclusions arising from the project.

2. Work prior to 1976

The project was started in 1974 (128-74-1 BIO UK), and during these first two years an organic scintillator spectrometer was developed. This consisted of a 1 cm diameter x 1 cm high stilbene scintillator on a 30 cm quartz light-pipe connected to a photomultiplier and a sophisticated pulse-shape discriminator (Harwell type 8850 ; Link Systems Model 5010). A suitable interface to a transportable multi-channel analyser (Canberra 8100) was also built and tested, and a computer program was written to deduce the neutron spectrum from the pulse height spectrum using a differentiation method.

At the same time, a mixed transport and diffusion program was written to treat the irradiation of a rectangular block of material by a plane-parallel beam of neutrons from an arbitrary direction. In this program the first few collisions of each neutron are solved algebraically using the full transport equation which takes explicit account of the directions of the neutrons as well as their energies. After two or three scatterings it is assumed that the angular distribution of the neutrons is isotropic, and the spectrum is used as a source for the numerical solution of a set of coupled diffusion equations corresponding to a set of energy intervals. The material is assumed to be a rectangular block with Cartesian lattice points at unequal intervals. The diffusion equations for the lattice points yield matrix equations which are solved with the use of sparse matrix techniques developed at AERE. The neutron spectrum is thus derived for each point of the three-dimensional lattice.

3. Work during the contract 1976-1980

3.1 Experimental work using the organic scintillator spectrometer

In the first programme of experimental work the organic scintillator spectrometer was tested with monoenergetic neutrons in the energy range 0.5-6 MeV to investigate the validity of the unfolding method and to obtain data on the (non-linear) relationship between the recoil proton energy and the light output of the scintillator. Experimental measurements were then made of the neutron spectra inside a 30 cm x 30 cm x 15 cm rectangular water phantom irradiated by a point source of neutrons incident on the larger face. Two incident energies (3 and 6 MeV) were used.

These results were compared with calculations (see section 3.2) in a paper presented at the IIIrd Symposium on Neutron Dosimetry in Biology and Medicine (1977)(6). Some additional work using 1 MeV neutrons and a spherical ^{3}He proportional counter spectrometer was also included in the paper, in which it was concluded that, because of its high sensitivity to low-energy neutrons, a ^{3}He spectrometer was unsuitable for in-phantom use.

Before the second programme of spectral measurements could be undertaken an investigation of the background neutrons was necessary, and the scintillator had to be replaced. A new series of response functions were then measured for neutron energies from 0.5-7.0 MeV. Various modifications to improve the unfolding program were also made. A least squares program based on GASP (7) was also tried as an alternative unfolding method for deducing the neutron spectrum from the pulse height spectrum; this gave disappointing results. Also it was determined that the spectrometer was unreliable below about 1.5 MeV because of the effect of the light-pipe; other considerations showed that for the energy range 1.5 MeV-9 MeV a program based on differentiation would be adequate. These conclusions, and a full description of the spectrometer are to be published as a Harwell report (8). Preliminary measurements with 14.8 MeV neutrons confirmed that the differentiation method of analysis would not be valid at this energy because of contributions to the response functions from other charged-particle reaction products (in addition to recoil protons).

Spectra inside the rectangular water phantom were measured for 3,5 and 7 MeV incident neutrons from a point source in front of the large face.

3.2 Calculations using the transport/diffusion program

Calculations were initially undertaken using the transport/diffusion program for plane-parallel beams of neutrons having energies of 3 and 6 MeV incident on the large face of a 30 x 30 x 15 cm rectangular water phantom. Comparison with the experimental measurements (6) was difficult because a plane-parallel beam could not be realised experimentally.

Improvements were subsequently made to the treatment of the oxygen cross-section which is oscillatory above 1 MeV, and to the number and separation of the energy points used by the program. To confirm the validity of the program, it was decided that it would have to be adapted to treat a point source of neutrons (at any distance) rather than a plane-parallel beam. This would also have a useful application in radiotherapy studies. These modifications have just been completed and the new program has now been used to calculate spectra in the phantom for 3,5 and 7 MeV neutrons incident from a point source as in the experimental studies. A folding program to broaden the calculated spectra to match the resolution of the spectrometer has also been written.

A comparison of these calculated spectra with those measured using the organic scintillator spectrometer will be made in 1981, outside the term of this contract.

3.3 Tests of the program to calculate dose and dose-equivalent from the neutron spectrum

The computer program which calculates dose and dose-equivalent from the neutron spectrum (4) can also be used to calculate event-size distributions in a Rossi counter (9). Event-size spectra have been measured using a Rossi counter irradiated by 2,3,5 and 16 MeV neutrons, and these have been compared with those predicted by the program. Discrepancies close to the peak in the proton event spectrum can only be explained by errors in the proton stopping powers or W-values used in the program. Larger discrepancies in the predicted and observed numbers and sizes of the heavy-ion events indicates substantial errors in heavy-ion cross-sections or wall stopping powers, and W-values or gas stopping powers. These observations were briefly reported at the European Dosimetry Group Meeting at Dundee (1979) and are being prepared for outside publication (10).

4. Conclusions

This work has shown that an organic scintillator spectrometer using a long light-pipe works quite satisfactorily, although with a relatively high energy-threshold. The transport/diffusion method of calculation has proved to be quite complex to set up. A full comparison between existing measured and calculated spectra will be made in 1981. There have been found to be some shortcomings in the input data (cross-sections, stopping-powers and W-values) in the Edwards and Dennis program (9), which require further investigation.

For future work it is recognised that realistic heterogeneous models of the human body are required. Such calculations are in principle possible using the present transport/diffusion program, as different compositions (including void) can be assigned to each point of the three-dimensional mesh. However, considerable development work would be required and it is thought that good statistical sampling codes with the necessary geometrical versatility already exist and are probably preferable.

The organic scintillator spectrometer is suitable for use in any phantom, although it will be exceedingly difficult to develop the tissue- and organ-equivalent materials to construct a realistic human phantom for experimental studies with neutrons, so further work with it is not envisaged in the near future.

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Title of project nr 3: The mutagenic and lethal effects of neutrons and γ-rays on mammalian cells

Head of Project and scientific staff: J C Asquith, P D Holt, A H Reading
J A B Gibson

Introduction

The main aim of this programme is to obtain experimental data on dose-response relationships for cellular radiobiological end-points which can be used to construct microdosimetric models of the effects (Project No 4). The models are utimately intended to allow extrapolation of high-dose data on the risk to man of carcinogenesis to the low doses and dose-rates of concern in radiological protection.

The cell lines used have been (i) a line of V79-4 Chinese hamster fibroblasts, (ii) L5178Y mouse lymphoma cells and (iii) three primary human lines, obtained from a foetus, an infant (foreskin sample) and an adult respectively. The radiations used have been gamma-rays (^{60}Co) and fast neutrons from the ^{9}Be(d,n) reaction, with a bombarding energy of 3 MeV, and also from ^{252}Cf. Some work has been carried out on mutagenesis and chromosome aberration but the greater part has been concerned with cell killing.

In order for the fitting of microdosimetric models to cellular radiobiological data to be useful it is necessary to know whether more than one mechanism contributes to an observed radiobiological endpoint, and, if so, to obtain the variation of the separate components of effect with dose and radiation quality. Thus much of the work has been carried out using synchronized cells since the effect observed in an asynchronous culture is a superposition of the effects at different stages of the cell cycle. A programme of work has also been carried out on the effect of the repair-inhibitor caffeine on survival.

Survival curves of synchronized cells

Survival curves of synchronized cells have been obtained for the Chinese hamster cells for both neutron and gamma-ray irradiation and for the mouse cells for neutron irradiation. The survival curves of the asynchronous mouse cells show a negative curvature, whereas those of the synchronized cells are all exponential. The sum of several different exponential curves will be a survival curve which has negative curvature when plotted on the usual logarithmic-linear scales. The survival curves of the Chinese hamster cells show a variation through the cycle for neutron irradiation which is qualitatively similar to that for gamma-ray irradiation, but less pronounced. For neutron irradiation in G2 the survival is exponential with dose. At other stages in the cycle the neutron survival shows a small shoulder (extrapolation number $\sim$2) whereas the gamma-ray survival shows a large shoulder at all stages (extrapolation number $\sim$20).

The effect of repair-inhibition by caffeine on cell survival

Extensive experiments were carried out on the effect of repair inhibition by post-irradiation treatment with caffeine in Chinese hamster cells. Following single-doses of gamma-rays caffeine increased the sensitivity but did not appear to alter the extrapolation number, whereas after single doses of neutrons the shoulder was removed entirely. In experiments in which caffeine was added at various times to cells irradiated at the G1/S boundary it was found that caffeine inhibited repair at two different times in the cell cycle, namely in early S-phase and in G2 phase.

A repair-saturation model of cell damage

The results of both the caffeine experiments and the synchrony experiments in Chinese hamster cells were tentatively explained by a model in which the shoulder on the survival curve results from the saturation of a repair system at high doses, causing an increase of radiosensitivty with dose. The sensitizing effect of caffeine was postulated to arise from its reduction in radiation-induced division delay, giving less time for the repair system to complete the repair of damage. On this model it is not necessary to assume the existence of "sub-lethal damage"; the sparing effect of fractionation can be accounted for by the recovery of the repair enzyme system in the split-dose interval. This model is supported by the survival curves obtained for the human cells; a shoulder was obtained for the adult line for both neutron and gamma-ray irradiation but no shoulder was obtained for the foetal cells for either type of radiation. The natural inference is that a repair system was operating in the adult cells which did not operate in the foetal cells.

Chromosome aberrations in Chinese hamster V79 cells

Dose-response relations were measured for chromosome aberrations in the Chinese hamster cells. In order to avoid the problem of the variation of radiosensitivity through the cell cycle, cells were irradiated in stationary phase where they are arrested in G1 and hence partially synchronised. The cells were scored for dicentries, centric rings, acentric fragments, chromatid gaps, chromosome gaps and breaks. Unexpectedly, it was found that the RBE of neutrons at low doses for the production of dicentrics and acentric fragments was only about 6, compared with the value of about 30 found by Lloyd, Purrott, Dolphin and Edwards (1976) for human lymphocytes. Dicentrics and acentric fragments would be expected to be lethal to the cell and graphs of the fraction of cells without these aberrations as a function of dose were very close to typical survival curves of this cell line, for both gamma-ray and neutron irradiation.

It seems likely therefore that, in these cells at least, killing can be accounted for by the production of gross chromosome changes, and a model for killing based on Neary's chromosome aberration model (see Project No 4) may be preferable to our tentative explanation in terms of the saturation of a repair system. The effect of caffeine on chromosome aberration production, however, has not been examined.

Conclusion

Extensive data have been obtained on survival curve slopes at different phases of the cell cycle for neutron and gamma-ray irradiation, and data have also been obtained on the effect of repair-inhibition by caffeine on cell survival. Chromosome-aberration dose-response curves for neutron and gamma-ray irradiation have been obtained in a line of cells for which there are already survival data, enabling the relationship between the two end points to be studied. Two models have been used to interpret the data; it has not been possible within this period to decide conclusively between them.

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Title of project nr 4: Microdosimetric models for the interpretation of mutation and cell killing in mammalian cells

Head of Project and scientific staff: P D Holt

Introduction

It is a common feature of cellular radiobiological effects, such as killing, chromosome aberration and mutagenesis, that the dose-response relationship is superlinear, i.e. that the response per unit dose increases with dose. The ultimate aim of this work is to understand the mechanisms responsible for radiation carcinogenesis, and the induction of heritable defects, sufficiently to be sure whether this superlinear dose-response relationship holds also for them. This question is of great importance for the nuclear industry because the answer affects very strongly the estimate of the risk to man of very low doses of radiation, since observations are available only at high doses and the low-dose estimate therefore has to be made by extrapolation.

During this period the field of study has been restricted to cell killing, mutagenesis and chromosome aberration. Experimental data have been obtained from this work (Project No 3) and from the literature. Two different models have been studied for superlinearity in dose-response: (i) The Dual-Action Model of Kellerer and Rossi (1972, 1978) and (ii) the exhaustible repair model (Alper 1977).

It has been shown (Holt, 1975) that the Dual-Action model is equivalent to Neary's (1965) chromosome aberration theory. It predicts that the dose-response relationship will be quadratic and that the linear, or "one-track" component will be proportional to LET; thus RBE values of 30-50 are predicted for neutrons with respect to gamma-rays; the RBE will decrease with increasing dose. From the ratio between the linear and dose-squared terms Kellerer and Rossi calculate an interaction distance and this comes out to be typically 0.5 μm. Thus the linear term in the response to very soft X-rays, where the range of the photo-electron is less than 0.5 μm, would be expected to be very small.

However this is not found to be the case, at least for cell killing and mutagenesis (Cox, Thacker and Goodhead, 1977). Further, the RBE values at low doses for cell killing and mutation are 5-10, instead of in the expected range 30-50.

The exhaustible repair theory of cell-killing

There are therefore difficulties in the way of accepting the Dual-Action Theory as a satisfactory account of killing and mutation in mammalian cells, and so consideration was given to the exhaustible-repair model. According to this model superlinearity is due to the exhaustion or saturation of a repair enzyme system. Caffeine is known to be a repair inhibitor and so the effects of post-irradiation treatment with caffeine on single-dose and split-dose killing in Chinese hamster (V79) cells were studied experimentally (Project No 3). It was possible to account qualitatively for the results by assuming that the sensitizing effect of caffeine was due to its reduction of division delay and hence of the time available for the repair system to act, and that this effect was more critical for neutron than for gamma-ray irradiation because of the much larger average number of lesions in a cell nucleus through which a charged particle had passed. Similar considerations would account for the experimental results on the variation of survival curve shape with the stage in the cell cycle at which the cells were irradiated, for both gamma-ray and neutron irradiation.

The chromosome aberration theory of cell-killing

However this exhaustible-repair model is not without difficulty since it is necessary to assume that the repair system is saturated by a very small number of lesions (less than 10). Further, experimental data from Project No 3 on chromosome aberration in Chinese hamster (V79) cells gave a neutron RBE similar to that found for cell killing, which is much lower than the neutron RBE's found for chromosome aberration in Tradescantia microspores (Neary, 1965) and in human lymphocytes (Lloyd, Purrott, Dolphin and Edwards, 1976), and so it appeared that virtually all the killing in V79 cells could be accounted for by the production of dicentric chromosomes and acentric fragments. For these two reasons renewed consideration was given to Neary's chromosome aberration theory, which is equivalent to the Dual-Action Theory. The sensitivity at low doses is predicted to be proportional to the LET only up to a certain value of LET and then to begin to fall. This value depends on the efficiency with which an energy-loss event produces a primary lesion in a chromosome, and this may vary (for example, due to the presence or absence of repair) between species. Thus a low value of this efficiency leads to the usual prediction of a low-dose RBE of 30-50 for neutrons with respect to gamma-rays, but a high value of the efficiency will lead to a lower value, such as is found experimentally for cell killing.

Neary's prediction of an interaction distance of ∿0.5 μm between primary lesions results from his assumption that this distance is the same when both lesions are produced by the same charged particle as when they are produced by two different charged particles. We assume, in the present work, following Virsik, Blohm, Hermann and Harder (1980) that a "fast" interaction between two lesions produced by the same particle occurs only if these are very close together. Interactions between lesions produced by different charged particles occur slowly after diffusion over much larger distances within the nucleus. It also appears that these slow long-range interactions have a low probability; repair of a lesion is much more likely than interaction.

Conclusion

It is concluded that cell killing in V79 cells is due almost entirely to chromosome aberrations and that both phenomena can be understood using a modification of the model of Neary and Kellerer and Rossi. This model should thus provide a useful frame-work for the correlation of data on killing and chromosome aberration in different cell lines and it is hoped that it will also be useful in interpreting data on mutation and malignant transformation. It remains, however, to see whether the experimental data on caffeine and on survival curve shape at different phases of the cell cycle can be incorporated into this chromosome-aberration model of cell killing. Thus we have not been able, within this period, to decide conclusively between the two models we have considered.

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Title of project nr 5: Track Parameters of Ionizing Radiation

Head of Project and scientific staff: M Marshall, T Budd, C S Kwok, D A Williams

1. Introduction

The cloud chamber is designed primarily to investigate the spatial distribution of ionisations in the tracks of charged particles. The aim is to produce data and parameters which are relevant to biological modelling (e.g. project 3) and microdosimetry (e.g. project 4).

Prior to 1976 photographs of the ionisation distributions produced by Al K X-rays in a convenient gas mixture had been obtained and converted to 3-D coordinates. Preliminary analysis of this limited data had been made to obtain a W-value and event size distributions. A tissue-equivalent (T.E.) gas mixture was being developed.

Work in the period 1976-80 is presented below.

2. Cloud Chamber Gas Mixtures

A modified nucleation theory has been developed which predicted that lower density (and therefore higher resolution) gas mixtures could be used in the chamber. The theory takes into account the bulk heating of the gas by the walls and the heating of droplets during growth. The predicted values of supersaturation required for condensation agree well with the experimental values for a T.E. gas and for water vapour.

The T.E. gas was chosen to have the same elemental composition (in H, O, C and N) as soft tissue and consists of water, ethanol, hydrogen, oxygen and nitrogen. The lowest density currently achievable is 9.5×10^{-3} kg m^{-3} after expansion which is a factor of six lower than for the original mixture. The magnification is 10^5 compared with unit density tissue.

There are difficulties in measuring the composition accurately. An infra-red spectroscopic technique has been developed to measure the partial pressures of the two vapours in the present mixture with an uncertainty of about 10%. This has involved extensive calibration since water vapour affects the alcohol absorption bands and oxygen affects the absorption of both water and alcohol. Together with other physico-chemical techniques (mass spectrometry and gas chromatography) this is used to determine the gas composition before and after a series of experiments.

3. Radiation

A simulated cloud chamber has been used to study the energy and intensity of radiations at the position of the active volume in the cloud chamber. A glow-discharge electron gun has been developed to produce carbon X-rays (284 eV) at the high intensity required. Aluminium and other X-rays are also generated by this method. For alpha particles (from a ^{244}Cm source) the energy and energy straggling after passing through various absorbers has been determined using a high resolution silicon diode (FWHM 19 keV) in the 'dummy' chamber. A collimating system with a fast photographic shutter was designed to introduce alpha particles into the chamber during the sensitive time.

4. Determination of Coordinates

A Quantimet-720 image analyser is now available and has been programmed to produce 2D coordinates rapidly. Interactive computer programs are being developed which will facilitate matching of orthogonal photographs to produce 3D coordinates.

5. Analysis of X-ray Data

Methods of analysis have been developed to enable Rossi 'Y' distributions and distributions of inter-droplet distances (proximity distributions) to be plotted separately for the various photo- and Auger electrons produced by aluminium K X-rays. Results for the non-T.E. gas mixture have been scaled for W-value and range and compared with theoretical data from Toulouse and Neuherberg. Satisfactory agreement has now been obtained after allowing for diffusion in the cloud chamber. Similar studies in the current T.E. gas mixture will be performed shortly.

W-values have been obtained in the various gas mixtures. In the present T.E. gas W-values for electrons from aluminium and carbon X-rays of (31.0±0.5) and (35.9±0.2) eV/ion pair have been obtained. These are similar to values obtained by other workers using different techniques.

6. Analysis of Alpha Particle Data

The coordinates of 38, 32 and 30 pairs of two orthogonal views of well focused track sections, each containing about 200 droplets and produced respectively by 5.0, 3.4 and 1.1 MeV alpha particles, have been obtained and are available to anyone interested.

Because of the high density of ionisations in alpha-particle tracks it is not generally possible to match sets of orthogonal data to obtain sets

of 3-D coordinates. Methods of analysis of the 2-D data to obtain microdosimetric distributions directly are being developed. Radial ionisation distributions have so far been obtained.

Differential W-values for alpha particles in the current mixture are 32.5±5.5 eV/ion pair at 4.98 MeV, 30.2±3.0 eV/ion pair at 3.46 MeV and 33.7±3.5 eV/ion pair at 1.14 MeV. The errors (1 std. dev.) are due mainly to uncertainties in the gas composition. A paper on W-values for the X-rays and alpha particles is being prepared.

7. Diffusion

A theoretical model to predict the effect of diffusion on the position of droplets condensed on positive ions has been derived. The average increase in separation between two ions is estimated to be about 200 μm in the chamber, in reasonable agreement with measured values.

8. Collaboration with Other Groups

Monte-Carlo data for the distribution of ionisations produced by electrons in air and water vapour has been obtained from colleagues at the University of Toulouse and at GSF, Neuherberg. Programs from both sources have been transferred to the Harwell computer. To facilitate comparisons with such data we have attempted to operate the cloud chamber on pure water vapour.

9. Water vapour

The operating conditions required to obtain satisfactory condensation of droplets have been calculated using the modified nucleation theory (Initial pressure 1.3 kPa at 14°C). A small number of alpha-particle tracks have been photographed but the tracks of low energy electrons have so far been unrecognisable. An instrumental fault is suspected and this work will be repeated when opportunity permits.

10. Conclusions

Theories have been developed to predict the supersaturation required for condensation in different gas mixtures in a cloud chamber and to determine the extent of diffusion. The chamber has been operated with a tissue-equivalent gas with a density six times less than that of the former mixture. Alpha particles and X-rays of appropriate energies and intensities can be produced during the sensitive time of the chamber. Coordinates of the droplet images can be measured automatically using an image analyser and 3D coordinates can be obtained using interactive computer programs.

Proximity distributions and 'Y' distributions have been obtained for ionisations produced by X-rays. Results have been compared with theoretical

distributions produced by colleagues in other laboratories. W-values have been produced for X-rays and alpha particles. Radial ionisation distributions have been obtained for alpha particle tracks.

Preliminary studies have been made on the operation of the chamber with water vapour alone.

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- Université Paul Sabatier, Service de Botanique et Biogégraphie, Toulouse, France
- Centre d'Etude de l'Energie Nucléaire, Mol, Belgique

CONTRAT N° : 272-79-1 BIO F, INRA, DIJON
273-79-1 BIO F, Université, TOULOUSE
274-79-1 BIO B, CEN, MOL

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- Marc A. DALEBROUX, Fonctionnaire Scientifique de la Commission, Coordonnateur du Programme
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THEME GENERAL DU CONTRAT :

Detection and assessment of biological effects of high natural radioactivity - Rapport Final.

PROJETS N° 1 et 3

Chefs des projets : Marcel DELPOUX
Hubert DULIEU, M.R. au C.N.R.S.

Collaborateurs scientifiques : Marc A. DALEBROUX
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Titre des projets : GENETIC EFFECTS OF HIGH NATURAL RADIOACTIVITY AND RADIOSENSITIVITY TESTS ON PLANTS AND PLANT CELLS IN/FROM AREAS WITH VARIOUS LEVELS OF NATURAL RADIOACTIVITY

Reminder :

In southwest France, there are uranous outcrops over which abnormally high radiation dose rates can be measured : up to 15 mrd/h., *i.e.*, 135 rd/y., whereas usual dose rates range between 0.010 and 0.035 mrd/h., *i.e.*, 90 and 300 mrd/y., approximately. It should be kept in mind, however, that these relatively high dose rates still lie within the infralethal range.

I - STUDIES ON PLANT SPECIES PRESENT ON THE OUTCROPS

I.1 - THE FLORA

The flora is adapted to submediterranean climatic conditions and to various regional soil characteristics. Among the numerous species that tolerate uranium in the substrate, no uranium-specific species was identified. However, careful observation showed that the distributions of certain species were somewhat affected by that of uranium (A.R. 1976, 316-317 ; A.R. 1977, 390-391 ; A.R. 1978, 401). A few cytological abnormalities were also observed in *Cerastium brachypetalum* Desp. (A.R. 1977, 391).

I.2 - RADIOSENSITIVITY OF PLANT SPECIES

Perennial plants living on radioactive sites, even delivering very low dose rates, do receive high total doses. In order to find out whether or not some adaptation could occur in the long run, seeds were collected on several species both from radioactive and control areas, and set to germinate. Seeds or plantlets were artificially irradiated at high doses by means of a Cs-137 source.

Plantlets of *Quercus pubescens* Willd. (A.R. 1977, 386 ; A.R. 1978, 396 ; A.R. 1979, 414) from seeds collected on a radioactive site and its close surroundings were more radiosensitive than control plantlets issued from seeds harvested in nonradioactive areas.

In *Aegilops ovata* L., seeds collected on a radioactive site were found to be less radiosensitive than those collected in a control area.

These results will be thoroughly discussed in future publications.

I.3 - ALBINISM IN *Galactites tomentosa* Moench.

In order to explain the presence of white capitula on plants that also carried normal lilac-colored capitula, it was suggested that mutation(s) had been induced by radiations emitted by the uranium present in the site on which these abnormal individuals were found (A.R. 1979, 415). This hypothesis was genetically verified, and detailed results will be presented in a future publication.

All these interesting data may be regarded as a confirmation of those obtained by DELPOUX (State Doctoral Thesis, University of Toulouse, 1974). Although suggesting that various biological and genetic effects can be attributed to the action of ionizing radiations, external and/or internal, produced by uranous outcrops, the findings mentioned above do not constitute a clear-cut demonstration. Only external irradiation, applied at the same dose rates to well known markers, can provide convincing evidence. This was the scope of the experiments described in Section II below.

II - IRRADIATION OF MATERIAL GROWN OVER URANOUS OUTCROPS OR DEVICES

Two marker systems were utilized.

II.1 - THE a_1^+/a_1 a_2^+/a_2 SYSTEM OF TOBACCO

This genetic system consists of a double heterozygote, a_1^+/a_1 a_2^+/a_2, of *Nicotiana tabacum* L. var. *xanthi* n.c.. The two loci are homeologous and involved in chloroplast differentiation. The mutant factor a_1 antagonizes both a_1^+ and a_2^+ whereas mutant a_2 is amorphous. The double heterozygote can be readily obtained by crossing line a_1^+/a_1^+ a_2/a_2, phenotypically normal, with partially chlorophyll deficient line a_1/a_1 a_2^+/a_2^+. The F_1 has greenish-yellow leaves. Either spontaneously or under the action of chemical or physical mutagens, the genetic composition of the system can be modified, yielding mostly reverted green cells ; the frequency of modifications to white and yellow cells is negligible and hence may be disregarded. As an individual grows, each reverted cell yields a clone that appears as a green spot in the palisade tissue

of the greenish-yellow leaf. Since a very large number of cells can be observed in one single individual (1000 to 2000 cells/mm^2 of leaf), this marker system is particularly handy for detecting effects of low and very low doses of radiations with very good statistical precision. The genetic effect of a given dose can be expressed as average reversion rate per cell cycle on the basis of the number of reverted cells or, equivalently, of reverted leaf area. It has been shown [Dulieu and Dalebroux, *Mutation Res.* 30(1975)63-70] or reminded [Fabries and Delpoux, *Mutation Res.* 49(1978)377-382] that, for a given individual, there exists a simple relationship between the total leaf area S and the reverted area S_g on one hand, and the reversion rate p on the other :

$$p = 1 - \left[\frac{S - S_g}{S}\right]^{1/t}$$

where t is the number of cell cycles that actually took place during the chronic irradiation, and, since spots smaller than 0.05 mm^2 cannot be detected by the observation method used (Fabries and Delpoux, 1978 above), is equal to

$$\frac{\log N}{\log 2} - 7.$$

N is the total number of cells observed that corresponds to the total leaf area S with cell density d (number of cells per unit area), so that N = dS.

It was shown that the reversion rate on plants grown over different uranous outcrops was significantly higher than that observed on individuals placed over inactive control sites (A.R. 1977, 384 ; A.R. 1979, 412-413). The study of the dose-response relationship was undertaken. For dose rates ranging between 0.3 and 0.4 mrd/h. as well as for higher rates ranging up to 1.00 rd/h., the response was found to be linear (A.R. 1976, 312-313 ; A.R. 1977, 385 ; A.R. 1978, 392-393) ; below the 0.3-0.4 mrd/h. range, no significant increase of the spontaneous reversion rate was observed (A.R. 1977, 385 ; A.R. 1978, 393-394). It should be pointed out that the effective dose received by the material is simply that delivered in 24 h. ; indeed, since continuously dividing

material is involved, the reversion of a given cell takes place during cell division, so that the effective irradiation period is at most equal to the duration of a cell cycle, which lasts about 24 h.

II.2 - THE *waxy* SYSTEM OF BARLEY

Locus *waxy* is involved in the synthesis of starch, the composition of which is modified when the wild-type gene mutates. Mutants are sought out in pollen populations of *Hordeum vulgare* var. *Monlon*. As is well known, wild-type pollen grains turn dark brown in the presence of iodine, whereas mutated grains turn red. Evaluation of the frequency of *waxy* mutants in pollen populations constitutes a mutagenicity test for various factors ; in this case, the effects of low chronic dose rates of ionizing radiations were investigated.

As for tobacco, it was repeatedly demonstrated that the frequency of *waxy* mutants in plants grown over uranous outcrops was significantly higher than that observed in individuals placed over control sites. The results obtained so far from studies undertaken to determine the shape of the dose-response curve are still incomplete. It seems that there is a linear response within the higher range of the low dose rates investigated up to now (A.R. 1976, 314 ; A.R. 1977, 385 ; A.R. 1978, 394-395). Below this range, it appears that the response is somewhat more complex and is being investigated (A.R. 1978, 395-396 ; A.R. 1979, 414).

III - INTERNAL IRRADIATION

Two experiments were carried out that consisted in growing tobacco and barley individuals directly in substrates with different radioactivity levels.

Tobacco was grown in three calcareous and one granitic substrates in Sault Region (Pays de Sault, Aude), characterized by dose rates of 0.010 mrd/h. (first three substrates) and 0.020 mrd/h., respectively.

The reversion rate observed on plants cultivated in the calcareous substrates was twice that on plants grown in the granitic soil (A.R. 1978, 394 ; A.R. 1979, 413).

The frequency of *waxy* mutant pollen grains significantly increased when barley was grown in soil originating from uranous rocks (A.R. 1976, 314).

IV - GENERAL CONCLUSIONS

IV.1 - FUNDAMENTAL INTEREST OF THE RESEARCH

The research carried out has contributed to a better knowledge of an unknown - or at least poorly known - component of the regional natural environment. Indeed, whereas a good deal of research has been performed on zones showing abnormally high radioactivity in India, Brazil, U.S.A., U.S.S.R., ..., uranous areas in southwest France, though well known by specialized geologists, were ignored by biologists and ecologists. The experiments performed allowed a better knowledge of them, especially from the dosimetry standpoint ; they also contributed to make a larger number of people realize that areas with really high natural radioactivity do exist in southwest France.

Also, through this research, it was possible to acquire information on genetic effects of low and very low dose rates of ionizing radiations delivered by uranous outcrops. Mainly by means of the a_1^+/a_1 a_2^+/a_2 system of tobacco and, to a lesser extent, the *waxy* locus of barley, reproducible and statistically reliable results were obtained. On their basis, it was possible to

- confirm and comfort the preliminary findings of DELPOUX (State Doctoral Thesis, University of Toulouse, 1974),
- assess and describe the dose-effect relationship at low and very low doses ranging down close to the zero point,
- reveal thresholds under which radioactivity seems to be ineffective.

These experiments also suggest the possibility of designing further research in order to

- assess the effects of internal irradiation by radionuclides incorporated in, or ingested by, living organisms,
- compare the effects of low and very low doses of ionizing radiations to those of other environmental factors (substrate, micro-climate, pollutants, etc.),
- assess the synergic effects, positive or negative, of the different factors mentioned above.

Finally, from a more general standpoint, since it was shown that low and very low doses of ionizing radiations emitted by active substrates do have genetic effects, it is not unreasonable to imagine that such factors, acting upon biological differentiation and speciation, can play an important role in those biological and biogeographical phenomena that are most fundamental for the diversification, renewal and development of the genetic potentialities of the biosphere.

From all these experiments, the role of low radioactivity in these processes could be evaluated without hazardous, or even unjustified, extrapolation made from results obtain with high and very high doses.

IV.2 - PRACTICAL INTEREST OF THE RESEARCH

The knowledge acquired on the basis of the results can be applied to problems related to radioprotection. So far they can help assess effects of low and very low doses of external irradiation, and thereafter contribute to the knowledge of the detriment caused by ionizing radiations. The experimental designs employed can be utilized to tackle the problems raised by the development of nuclear energy, like that consisting in validating, on experimental grounds, the norms of radioprotection and so contribute to the nuclear safety in and around power and industrial plants using atomic energy.

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IN PREPARATION

DELPOUX M. and M. DALEBROUX. Study on Radiosensitivity of Pubescent Oak (*Quercus pubescens* Willd) in Areas with High Natural Radioactivity.

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PROJET N° 2
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Chef du projet : Alain LEONARD
Collaborateurs scientifiques : G. Decat, E.D. Léonard.
Titre du projet : CYTOGENETIC INVESTIGATIONS ON MAMMALS EXPOSED TO HIGH NATURAL RADIOACTIVITY.

It is generally difficult, or even impossible, to determine the exact doses of radiation received by humans or animals living in regions of high natural radioactivity. This explains why genetic and cytogenetic investigations performed so far on animal species have yielded conflicting results. The present study was carried out on laboratory animals maintained in captivity over "hot spots" near Lodève in southwest France, where the dose rate from external terrestrial sources can reach 100 rd/y. Under such conditions, it was possible to accurately determine the exposure dose and so compare the data with adequate controls as well as with laboratory experiments.

Over a site where the dose rate amounts to about 8 mrd/h., a hut was built on the floor of which cages with laboratory rabbits were placed. The Gamma doses were determined by placing an individual lithium fluoride dosimeter around the neck of each animal. The rabbits were kept in the hut for a 28-month period and blood samples were taken after 4, 8, 12, 16, 20 and 28 months in order to study chromosomal aberrations in somatic cells (lymphocytes). Air samples inside the hut were taken in spring and fall and the ^{222}Rn contents measured. In order to find out whether ^{222}Rn and its daughter products could participate in the production of chromosome aberrations, model experiments were performed under controlled conditions of radon exposure. Induction of chromosome aberrations in male germ cells was studied in mice placed in the hut during summer, a longer stay being impossible due to the sensitivity of mice to climatic variations. Control animals were kept near the radioactive site as well as in the laboratory.

The dose of γ rays received by the rabbits during the 28-month period varied from 43 up to 163 rd, while that of the control was 0.3 rd. The incidence of unstable chromosome aberrations, such as fragments and dicentrics, increased during 8 months, but was very variable thereafter. It is likely that the lack of correlation between dose and chromosome aberrations is partly due to the very short half-life of rabbit lymphocytes. Model experiments with rabbits whole-body irradiated with 300 rd X-irradiation showed, indeed, that the numbers of dicentrics and chromosome fragments decrease with time after exposure. A plot of log-abnormalities against time suggests a single exponential decline $A(t) = A_o \exp (- t/1.359\ T_{1/2})$, where A is the percentage of aberrations as a function of time, A_o is the percentage of aberrations at time t_o, and $T_{1/2}$ is the half-life time of aberrations, for dicentrics and fragments, although there are somewhat more aberrations than expected left after long periods of time. The half-life time of dicentrics and fragments were not very different from one another (70 and 46 days), so that a slope could be calculated for both types of aberration together by nonlinear regression, yielding a half-life time of 58 ± 14 days for time ranging from 2 h. to 140 days. Consequently, one should expect the level of aberrations to quickly reach a plateau. Most likely, the large variations observed after 8 months resulted from death of lymphocytes which also caused lymphopenia and a reduced response to mitogens ; this kept us from observing a sufficiently large number of mitoses.

In the model experiments with radon exposure, five male rabbits were exposed to a total of 10.66 WLM (Working Level Month) of radon split into 13 exposures of 15 to 20 h. distributed over one month. One working level month (WLM) corresponds to an exposure of 170 h./month. The rabbits in the cages at Lodève received a continuous exposure of 0.27 WL from short-lived radon daughter products, i.e., 0.27 x 4 x 12 = 12 WLM during one year. The model experiments were, therefore, carried out with 10.66 WLM since maximal chromosome aberrations were observed after 8 months. Since no chromosome aberrations were detected in the lymphocytes from those animals, it should be concluded that the chromosome aberrations observed in the rabbits maintained in Lodève were not due to the radon exposure but essentially to gamma irradiation.

The three series of experiments performed on male and female mice kept in the hut during the summer period demonstrated that doses of 10 to 70 rd of gamma radiation delivered at low rate influenced the subsequent fertility of the exposed animals, the number of offspring being higher when the males were exposed and lower if the females were irradiated.

The following conclusions can be drawn :

1. Relatively low doses of gamma radiation delivered at low rates (± 8 mrd/h.)
   a) increase the frequency of chromosomal aberrations in the somatic cells of rabbits ;
   b) increase the fertility of male mice whereas that of the females is reduced.
2. Radon 222 and its daughter products are not responsible for the chromosome aberrations observed in rabbits maintained in an area of high natural radioactivity.
3. Small mammals such as rabbits cannot be considered as a good model for biological dosimetry based on chromosome aberrations induced in peripheral blood lymphocytes.

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Contractor : Carlsberg Laboratory, Department of Physiology

Contract No. : 202-76-1 BIO DK

Head of the Research Team : Professor Diter von Wettstein

General Subject of Contracts : Chromosome pairing and chiasma formation in human meiosis

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Title of the Project : Chromosome pairing and chiasma formation in human meiosis

Head of Project and Scientific Staff : Prof. Dr. Diter von Wettstein
Lic.scient. Preben Bach Holm
Lic.scient. Søren W. Rasmussen

The assessment of the effects of radiations and radiomimetic agents on human male and female meiosis, i.e., on genetic recombination and chromosome disjunction requires a detailed knowledge of chromosome pairing and chiasma formation at the ultrastructural level. It has been the purpose of the present research project to analyze chromosome pairing as mediated by the synaptonemal complex at zygotene-pachytene and chiasma formation from the synaptonemal complex at diplotene in primary spermatocytes. Meiotic nuclei at the relevant stages have been sectioned serially, electron micrographs of these sections taken, and three dimensional reconstructions for all bivalents prepared with the specific aim to count recombination nodules as well as the number of incipient chiasmata recognizable at early diplotene by short retained pieces of synaptonemal complex. The number of nodules and chiasmata determined in this way has been compared with the number of chiasmata countable at late diplotene, diakinesis and metaphase I in the light microscope.

To date 76 nuclei from 6 human males have been analyzed comprising leptotene, zygotene, pachytene, metaphase I, and anaphase I. At leptotene a lateral component is organized along each chromosome and the telomeres attach to the nuclear envelope. At early zygotene the attachment sites aggregate and a bouquet is formed. Synaptonemal

complex formation is in most cases initiated from both ends of the homologues. Pairing of the short arms of the acrocentric bivalents and the X and Y chromosomes is delayed compared to the remainder of the genome. Prior to and during pairing homologous regions are frequently widely separated, hence demonstrating the absence of presynaptic alignment of homologues in human spermatocytes.

Sixty percent of the analyzed late zygotene nuclei contained one or more chromosome or bivalent interlockings. It has been unequivocally demonstrated that these interlockings are resolved by breakage followed by a precise reunion of the broken ends. A comparative study of Bombyx spermatocytes have confirmed that breakage and reunion of chromosomes and bivalents are naturally occurring phenomena during chromosome pairing. At early pachytene all bivalents are fully paired with continuous synaptonemal complexes.

Present evidence assignes meiotic recombination to be associated with electron dense nodes, socalled recombination nodules, attached to the central region of the synaptonemal complex. In human spermatocytes recombination nodules are present from early zygotene. At late zygotene, an average number of 101 nodules per nucleus is found. Assuming that all regions of the complex have equal probabilities of receiving a nodule about 144 nodules are expected to be present at the moment of complete pairing. At early pachytene the mean number of nodules is 75. Generally, nodules appear to be distributed at random among and along the bivalents. Nodules are present, however, in excess in the telomere regions and in the XY bivalent, while the centromere region, the secondary constrictions, and the short arms of the acrocentric bivalents are relatively depleted of nodules. In these regions the nodule distribution is similar to the chiasma distribution at diakinesis. For the remaining regions differences between the two stages are revealed, probably signifying terminalization of the chiasmata. An analysis of the number and distribution of meiotic recombinational events is thus feasible at the ultrastructural level and allows also a prediction of the level of nondisjunction in human spermatocytes. This is illustrated by the agreement between the observed number of sex bivalents without recombination nodules and the frequency of precocious separation of the sex chromosomes at diakinesis. Absense of nodules is pre-

valent among the shorter bivalents, in particular the acrocentric bivalents. Hence these are expected to be more often involved in non-disjunction than the other autosomes.

During pachytene a progressive condensation of the chromatin takes place, particularly prominent in the centromeric regions of all chromosomes and in the secondary constrictions of chromosomes 1, 9, and 16. These chromatin condensations provide suitable chromosomal markers which in combination with absolute length measurements permit the identification of individual bivalents. The relative length of individual chromosomes as determined from the synaptonemal complex length was compared with the relative length of diakinesis bivalents and somatic metaphase chromosomes as determined by light microscopy. Good agreement is obtained and allows in combination with the markers an identification of 14 of the autosomal bivalents, while the remaining 8 bivalents can be assigned to the C and D groups.

The potential of the technique of serial sectioning and three dimensional reconstruction for analyzing chromosomal rearrangements has been assessed. A translocation, which by light microscopy was judged to be non-reciprocal (46, XY, t (5p-; 22p+)), has been investigated. At early pachytene, a quadrivalent is present with a synaptonemal complex between the short arm of the normal chromosome 5 and the segment translocated to the short arm of chromosome 22. At mid pachytene the pairing specificity is suppressed and the segment translocated onto chromosome 22 may engage in non-homologous associations. The telomere region of the translocation chromosome 5 was observed to consist of a 200 nm region of condensed chromatin. This region is recognized as the telomere region of the short arm of chromosome 22, revealing that a reciprocal translocation has occurred.

Three dimensional reconstructions of prometaphase I, metaphase I and anaphase I cells have been performed. At prometaphase I, fragments of synaptonemal complexes are present within the bivalents. At metaphase I, these fragments are expelled from the bivalents and at early anaphase I, the bivalents are almost completely devoid of fragments.

Further studies will show whether in human spermatocytes these synaptonemal complex chiasmata at metaphase I substitute for the chromatin chiasmata observed in other organisms at this stage or whether they represent an additional safety device which insures a regular disjunction.

Cand.med. J.G. Berthelsen has analyzed the effects of different hormones on the ultrastructure and behaviour of meiotic prophase chromosomes using biopsies taken in a project dealing with the search for a male contraceptive. Pachytene spermatocyte nuclei exposed to long-term treatment with d-norgestrel and testosterone enanthate have been reconstructed in a feasibility study of the use of this technique in assessing the effect of radiation and cytostatica on the formation of the synaptonemal complex and the evolvement of chiasmata. No damage was revealed by the hormonal treatment in comparison to the reference material. The technique was found usable for the purpose. Seventy five biopsies from patients with testis cancer prior to treatment with radiation and cytostatica have been fixed and embedded. New biopsies will be taken at the end of the treatment and constitute a material for the analysis of long-term effects of radiation on gonads.

To gain more insight into the two phase pairing system previously reported in triploid Bombyx oocytes and in a human translocation heterozygote, chromosome pairing and synaptonemal complex formation has been analyzed in tetraploid oocytes of Bombyx mori in which crossing over and chiasma formation is lacking. At early pachytene, quadrivalents are frequent whereas at mid-late pachytene, most of the quadrivalents have resolved into bivalents. The process of pairing and synaptonemal complex formation thus consists of two distinct phases: 1) a specific zygotene pairing with synaptonemal complex formation restricted to homologous chromosome regions and 2) a correction of irregularities in this pairing by partial dissolution of the central region of the synaptonemal complex succeded by a second round of synaptonemal complex formation.

Since a time course interpretation of the ultrastructural data on diplotene, diakinesis and prometaphase in human spermatocytes at present is not possible, inter alia because of lack of suitable reference material in any other organism, chiasma formation has been studied in the male Bombyx. In this organism, chiasmata evolve in bivalent regions which at mid-late pachytene contain a large recombination nodule. By regional chromatin condensation around the nodule, a domain of condensed chromatin forms during diplotene, at first in association with a retained synaptonemal complex fragment, later by formation of a complex circular structure which in combination with two regions of condensed chromatin gives the chiasma a tripartite appearance. At late diakinesis, the number of chiasmata decreases and by metaphase I very few remain.

In conclusion, the research plan outlined in the original proposal has been followed and the major goal achieved, namely to provide detailed knowledge of chromosome pairing and chiasma formation at the ultrastructural level in human spermatocytes. It has also been shown that the technique is suited for the analysis of long-term effects of cytostatica and radiation. The similarity of the meiotic prophase at the ultrastructural level in Bombyx and Homo renders the analysis of for example the short-term effects of radiation in Bombyx relevant also for human spermatocytes.

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Contractor :	The Finsen Laboratory, Copenhagen.
Contract N°	203-76-1 BIO-DK
Head of the research team :	Mogens Faber
General subject of contract :	Radiation Sensitivity of the Human Ovary

Title of Project n° 1	:	Radiation Sensitivity of the Human Ovary	
Head of project and scientific staff	:	Solvejg Bager	Birgitte Højager
		Ruth Himelstein Braw	Sue Lintern-Moore
		Anne Grete Byskov	Jørgen Lippert
		Mogens Faber	Philip Moore
		Jørgen Grinsted	

1. COLLECTION OF HUMAN OVARIES

Since 1964 human ovaries have been collected from autopsies and operations at various hospitals in Denmark, Great Britain, Switzerland, Israel and U.S.A. Approximately 2300 ovaries from fetuses, children and adults with information about diagnosis and treatment (radiation, cytotoxic treatment etc.) which have now been registrated systematically at a computer.

a. The normal development was studied on ovaries received from the coroner's office of autopsies performed on young women who died a violent death or after a short illness.

b. Fresh ovaries were received after surgival removal (usually during treatment of breast cancer). Follicles from these ovaries were used to study growth and atresia by assaying the hormonal content of the follicle fluid and the variation in the DNA content in the granulosa cells by flow cytometry.

c. Fetal gonads prepared from abortion material were received from the University Hospital of Copenhagen. The gonads were cultured for different time with and without hormones to serve as a model for studying the radiation effect on growth and differentiation of fetal ovaries.

2. THE NORMAL DEVELOPMENT OF THE HUMAN OVARY

The differentiation and growth of the oocyte and the follicle has been described during fetal life, childhood and in the adult woman. We have defined the early follicle formation which start already in the fetus, the progressive stages of follicle development and atresia during infancy and the events which last to the first ovulation. These studies enable us to define changes of the ovary after different influences as radiation, diseases and drugs.

Attempts to define the spatial relationship between follicular growth and atresia have been initiated but so far without definite conclusion although there are indications of centers growth or atresia.

3. THE DEVELOPMENT OF THE OVARY AFTER RADIATION

The infant ovary is particular sensitive to radiation and therapeutic radiation during childhood with the ovaries in the field causes severe damage. Follicle growth is inhibited and the number of small follicles is in most cases reduced when examined 1 week to 12 months after irradiation.

In order to evaluate the long term effect of therapeutic irradiation with the ovaries in the field information on all patients in Denmark who have been treated for Wilms tumor was obtained. Only few ovaries have been available. All known cases have been traced and the gynecological history was studied. The menarche started at the expected time, but none of the children were yet at an age where a possible sterility was recognizable.

It appears quite evident that further studies on radiation effects of human ovaries after therapeutic treatment of malignant diseases can give no quantitative information. During the last lo years practically all tumor patients have been treated both with radiation and chemotherapy.

4. THE ABNORMAL OVARY

Spontaneous abnormalities had to be studied before it was possible quantitatively to evaluate radiation effect.

In some diseases the growth pattern and differentiation appear to be disturbed in a way not dissimilar to radiation effects. That is the case in Down's syndrome. It was shown that both the pool of non-growing small oocytes but also the larger ones are smaller than in normal ovaries.

5. CHEMOTHERAPEUTIC INFLUENCES ON THE OVARY

Ovaries from children with leukemia, that had received cytotoxic drugs for varying length of time were examined. Those drugs caused damage to all ovaries except in cases where drugs had only been used for a very short time (1 week).

The ovary collection has also been useful in studies of a number of non-radiological problems in a more quantitative way, as f.i. hormonal anticonception. It has been claimed that follicular growth was inhibited by this treatment, findings which we can not confirm.

6. EXPERIMENTAL APPROACHES

In studies on mice we have found that gonadotrophins protect large, growing follicles from becoming atretic.

About 95% of the total number of follicles in the mouse ovary are small, non-growing follicles.These follicles are extremely sensitive to radiation. It was therefore tested whether the small follicle in the irradiated ovary could be protected against atresia by injection of gonadotrophin. Irradiating mice at the age of 14 days and treatment with gonadotrophin (PMSG) in the dosis administered had no protective or healing effect on radiation damage in small follicles.

Other hormones, f.i. testosterone, are said to induce follicular atresia. Treatment of 21 days old mice with physiological doses of testosterone showed however, that the percentage of atretic follicles was not influenced; but the start of growth of small follicles was accelerated.

Attempts to extend the studies by use of monkeys as the nearest analogue to human ovaries failed due to lack of monkeys in most of the primate facilities which we have approaced.

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Temperature measurements of rabbit antral follicles.
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Contractor: Università di Roma

Contract nr.: 160-76-I-BIOI
Head of the research team: Prof. P.M. Fasella

General subject of contracts: Repair nucleases

Title of the project nr. 1
The ATP-dependent DNAase of Micrococcus lysodeikticus

Head of Project and scientific staff:
E.P. Whitehead (Commission)
G. Cerio-Ventura, F. Palitti, C. Salerno, A. Giartosio, P.M. Fasella.

The contract research has focussed particularly on the mechanism of the DNA-dependent ATPase/ATP-dependent DNAase of Micrococcus lysodeikticus, especially the processivity and the role of ATP analysed by kinetic methods.

In a study of the steady-state kinetics we found a surprising Michaelian dependence of velocity on DNA concentration (1). Other workers have since found a similar effect with E. coli enzyme. A novel type of kinetic analysis of the detailed dependence of velocity on ATP concentration and DNA length supported a processive mechanism, with formation of the enzyme-DNA complex being ATP-dependent. (1) In the same publication a new method of purification of the enzyme was described. The mechanism has been further analysed by studies of the randomisation of the enzyme molecule on molecules of DNA, studied by mixing at appropriate times radioactively labelled and nonradioactive DNA in incubations with the

enzyme. The results (2-7) have given direct evidence for the hypotheses made in (1), in particular of the processivity on double-stranded DNA and the ATP-dependence of the enzyme-DNA complex formation step. This step is slow but is essentially irreversible in the presence of ATP-Mg and may involve ATP hydrolysis since nonhydrolysable ATP analogues have no effect on either formation or stability of the complex. The action of the enzyme on single-stranded DNA on the other hand is not processive. Processivity is probably an important aspect of the participation of this and other enzymes in the repair process, as it could be a mechanism enabling the enzymes to find efficiently their way to the site of repair.

Tests of the enzyme with synthetic homopolydeoxynucleotide have shown little or no specificity with regard to bases. Recently we have worked with pyridoxal phosphate and derivatives of this synthesised here with a view to obtaining specific-site-directed inhibitors for various of the sites of the enzyme. Preliminary results (unpublished) have been encouraging.

During the contract period we undertook a review of all the DNA-dependent ATPases (8,9) emphasizing the unity of the mechanistic principles of these enzymes, and the close co-operation of the DNA-dependent activity with other enzymic activities such as polymerases, nucleases, ligases, methylases and proteases.

Other work has concerned the mathematical analysis of complex interactions in models of enzymes with particular reference to allosteric control. Important results achieved are: the simplification of forms and derivations of steady-state equations for complex mechanisms (10) and their application in steady-state analysis (11, 12); a novel graph-theoretical algorithm for analysis of steady-state properties (13-15) an analysis of macroscopic criteria of co-operativity (16) and of the microscopic conditions for this

(17) which has given for the first time the full conditions for every possible type of co-operativity in tetrameric proteins; and the relation of protein symmetry to co-operativity (18).

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Contractor : The Polytechnic of Central London

Contract nr: 168-76-1 BIO UK
Head of the research team(s): Professor G Holt

General subject of contracts:

Assessment and analysis of genetic damage in eukaryotes

Title of the project nr. 1. The use of model systems in Aspergillus nidulans and Chinese hamster cells for the detection of genetic damage induced by irradiation.

Head of Project and scientific staff:

Dr P Cohn, Ms. R Chambers, Ms. J Wood, Mr C Igwe

In this study the eukaryote Aspergillus nidulans was used to estimate the degree of chromosomal non-disjunction, mitotic crossing over, chromosomal deletion, gene conversion and point mutation caused by ionising radiation of different quality including fast electrons, soft X-rays, β-particles and α-particles. Depending on the genetical end-point being considered, the calculated RBE values for the raditions were markedly different. For example, following α-irradiation, the RBE values varied from 1.69 for mitotic crossing-over to 150.36 for non-disjunction.

With respect to chemical modification of the response of A.nidulans to radio-induced genetic damage studies have been made with the phorbol esters, 12-O-tetradecanoylphorbol-13-acetate (TPA), a tumour promoter, and its inactive (i.e. non-tumour promoting) derivative phorbol-13-acetate (PA) and two radiosensitisers, metronidazole and misonidazole. Conflicting data exist on the ability of TPA to induce sister chromatid exchanges in mammalian cells after initiation by mutagens. Our study has shown that while TPA and PA have no effect on the viability of the cells TPA, but not PA, increases the frequency of gene conversion after initiation by Co-60 γ-rays. Moreover, no such increase in the frequency of point mutation was found with either TPA or PA.

In terms of radiosensitisation to electrons the enhancement ratios for misonidazole were always higher than those of metronidazole and varied from 1.64 for crossing-over on linkage group I to 2.96 for crossing-over on linkage group VIII, compared with 1.16 and 2.42 for metronidazole. The oxygen enhancement ratio again depended on the particular genetical system being assayed and varied from between 2.43 for non-disjunction and 8.33 for gene conversion.

Work has progressed to establish a system in mammalian cells, which will demonstrate the genetic consequences of mitotic recombination at the X-linked hypoxanthine guanine phosphoribosyl transferase (HGPRT) locus. To date, mitotic recombination in mammalian cells has only been demonstrated cytotogically and biochemically.

The single copy of the HGPRT gene in V79 cells allows the effective production of a diploid locus, when two such cells are fused. Non-complementing and independently derived variants at the HGPRT locus may thus be selected for, using a forward selective system, resistance to 6-thioguanine (6TG), an analogue of guanine, whose intermediate products are lethal when 6TG is taken up by cells possessing normal or near normal HGPRT activity. Conversely the complete or partial presence of a wild-type phenotype may be selected for, with hypoxanthine, azaserine, thymidine (HAT) medium, a system permitting back selection. Azaserine blocks de novo synthesis, allowing only cells with active HGPRT to survive, since these cells can utilize hypoxanthine and thymidine as exogenous purine and pyrimidine sources. The introduction of autosomal markers into the parental cell types before fusion, allows the isolation of biparental hybrid cells, without subjecting the HGPRT locus to undue selective pressures.

The protocol for selection of the parental cell types was a follows. A subline was isolated from the male, established Chinese hamster lung line, V79, to ensure that ensuing treatments were administered to an isogenic population. Following ethyl methane sulphonate (EMS) mutagenesis, wild-type cells were challenged with ouabain (Oua), hydroxyurea or cycloleucine. Mutant clones resistant to any one of these compounds by single step selection were isolated, but only clones resistant to Oua remained stable, and were taken to constitute a parental stock, type I. These cells were treated with one of three mutagens: EMS, inducing predominantly point mutations; ICR-191, inducing predominantly frameshift mutations; or 3H-thymidine inducing predominantly chromosome breaks, before being challenged at 37^{o} with 5µg ml^{-1} 6TG. By electrophoretic and chromatographic characterization and comparison with wild-type cells, 6TG^{r} clones possessing approximately 5% of the wild-type enzyme activity were isolated.

$$\text{Parental type I : Oua}^{R}\ 6TG^{r}\ (5\%\ \text{HGPRT})$$

An alternative marker or system allowing the identification of a second parental cell type was sought. Following EMS, ICR-191 or ^{3}H-thymidine mutagenesis, wild-type cells were challenged at 39^{o}C with 5µg ml^{-1} 6TG. Resistant cells (6TG^{r}) are usually sensitive to HAT medium, but it was thought some might be HAT-resistant if their HGPRT activity was temperature sensitive. Of the 6TG^{r} clones isolated at 39^{o}C, 3% continued to proliferate in HAT-medium at 34^{o}C. Subsequent testing showed that these cells were also able to proliferate in HAT-medium at 39^{o}C, and 6TG-containing medium at 34^{o}C. The HGPRT of these cells was not temperature sensitive, therefore they were not suitable as the second parental type. In view of the few suitable dominant or codominant markers available, a two step selection procedure was used to isolate cells resistant to 1mM hydroxyurea (Hu^{R}). Following EMS mutagenesis, 6TG^{r} cells possessing approximately 5% of wild-type HGPRT activity were cloned to comprise the second parental stock, type II.

Parental type II : Oua^{S} Hu^{R} $6TG^{r}$ (5% HGPRT)

Parental types I and II were fused by treatment with polyethylene glycol. By plating in the presence of Oua and Hu, biparental hybrids were selected for. The presence of tetraploid cells may be confirmed by karyotype analysis.

By determining the frequency of restoration of HGPRT activity following the induction of crossing-over by ionizing radiations and chemical agents, these cells will serve as a system to study intragenic recombination.

Recent Publications

Normansell, I.D. and Holt, G. (1978) "The induction of chromosomal deletions in Aspergillus nidulans by various ionizing radiations". International Journal of Radiation Biology, 34, 553.

Normansell, I.D. and Holt, G. (1979) "The ability of ionizing radiations of different LET to induce chromosomal deletions in Aspergillus nidulans". Mutation Research, 59, 167

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Holt, G., Normansell, I.D., Wood, J.T., Igwe, C.N. and Chambers, R. (1979) "Studies on recombination at mitosis in eukaryotes" in 1978 Annual Report of Biology Programme - Health Protection. Commission of the European Communities. EUR 6263 417

Holt, G., Cohn, P., Chambers, R.J., Igwe, C. and Wood, J.T. (1980) "The use of model systems in Aspergillus nidulans and Chinese hamster cell lines for the detection of genetic damage induced by irradiation", in 1979 Report of the Programme "Radiation Protection" Harwood Academic Publishers for the Commission of the European Communities, EUR 6766, 433

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CONTRACTOR: College of Technology, Kevin Street, Dublin 8.

CONTRACT NO: 183/76/1 BIO EIR

HEAD OF RESEARCH TERMS: J.F. Malone.

GENERAL SUBJECT: Influence of agents encountered in normal physiology on the capacity of cells to repair and recover from radiation damage.

The project set out to investigate the radiobiological effects of physiological substances with a view to applying any significant effects observed to clinical and mechanistic studies of radiation damage and repair. Substances investigated include Vitamin A, Vitamin C, cAMP, Ethanol, Oxygen Deprivation, Lactate and Glucose metabolism. The results confirm the idea that useful radiobiological modifiers can be found among the relatively non-toxic physiological substances. It has also identified several new effects and added to the literature on previously known modifiers.

From the detailed results can be seen that the cellular metabolic state has a considerable influence on the radiation response of the cell. Work with oxygen deprivation, lactate and glucose metabolism reveals effects which could influence the interpretation of the oxygen effect, tumour radiobiology, as well as pointing to the importance of culture conditions in radiobiological experiments based on tissue culture systems. The work with Vitamin A, cAMP and Ethanol could suggest membrane involvement in radiation response since these substances all effect the membrane stability, but new results showing influences of these substances on the nucleus must leave the matter in doubt.

The curve fitting portion of the project involved writing a suite of programmes to provide Maximum Likehood estimates of the parameters of commonly used radiobiological models. The goodness of fit of data to the models was quantified and and index which could be used to discriminate between models was developed. A second phase of the project was concerned with optimising experimental design to obtain the most useful data to fit dose-effect curves in the required region.

RESULTS OF PROJECT NO. I

HEAD OF PROJECT AND SCIENTIFIC STAFF: J.F. Malone, C. Mothersill, M. Moriarty, J.K. Taaffe, A. Murphy, C. Seymor, M.K. O'Connor

TITLE: Influence of agents encountered in normal physiology on the capacity of cells to repair and recover from radiation damage.

GENERAL

The project commenced in 1976 and continued through to December, 1980

The major aims were (a) to investigate the radiobiological effect of physiological substances which generally have well documented effects on cellular metabolism with a view to elucidating the mechanisms involved in radiation damage and repair and (b) to study the possibility of using these substances in the management of victims of acute radiation accidents, radiotherapy patients and patients subjected to frequent diagnostic radiography. It was hoped that the use of physiological substances would permit more manipulation of concentrations and a more informed approach to mechanistic studies than the use of cytotoxic drugs whose effect on cellular metabolism is both more severe and less well understood.

The results over the last five years show that without doubt, physiological agents can alter the radiobiological response of mammalian cells. No clear mechanisms emerged but the results lend further support to some established ideas regarding the expression of radiation damage. These include the relevance of membranes and cell energy metabolism. As a direct result of the project, clinical trials were established to investigate the levels of Vit.C in the blood, tumours and normal tissues of radiotheraph patients. Trials were also established using a Vit. A analogue - Tigison and the Vit. A levels in the blood of patients is being monitored. The results may also have contributed to an understanding of the radiobiology of hypoxic tumours.

RESULTS IN DETAIL

The following substances or conditions were investigated: Vitamin C, Vitamin A, hydrocortisone, CAMP elevating agents, Ethanol, Lactate, Oxygen deprivation, glucose analogues and glycolysis inhibitors.

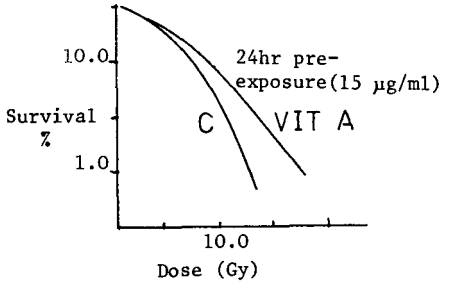
VITAMIN C.

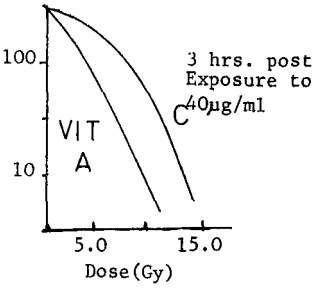
Vitamin C was found to be radioprotective when present during irradiation, the effect was seen as an increase in the D_o of the survival curve. Because of the nature of Vitamin C and published reports the mechanism is thought to be radical scavenging. This was the first substance investigated and has led to the most

follow up work so far. Studies on levels of Vitamin C in patients with tumours indicated that Vitamin C was accumulated by tumours but low in surrounding tissues. This fact could promote radioresistance in tumours.

VITAMIN A

An extensive investigation of the complex radiobiological effects of Vitamin A was performed. Exposure of cells for 24 hrs. to low levels of the Vitamin proved radioprotective, with an increase in the D_0 of the survival curve. High levels of Vitamin A reduced the survival curve shoulder and inhibited recovery of cells when given immediately before irradiation and during the recovery period. Long term exposure to high levels of the Vitamin reduced both the D_0 and the shoulder of the curve but the Vitamin was so toxic under these conditions that interpretation of the results is difficult. Neither of the Vitamin A analogues (Retinoic acid or Tigison, a phenyl derivative) had any radiobiological effect. Fig. I shows the two most useful radiobiological effects of Vitamin A. Clinical work with Vitamin A has commenced.





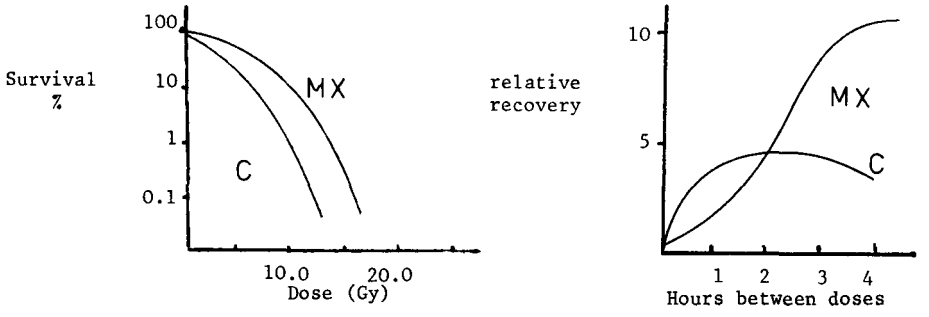
HYDROCORTISONE

Experiments with hydrocortisone under a wide range of conditions showed no radiobiological effect.

cAMP

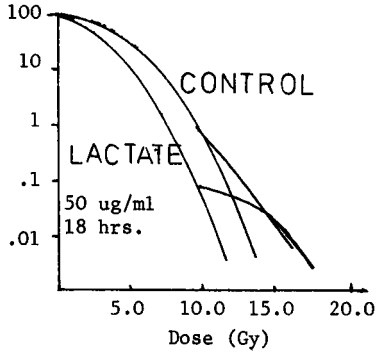
Several agents which raise or lower cAMP in cells were tested. The most effective were prostaglandin E and Isobutyl Methylxanthine. Long exposure(24 hrs.) prior to irradiation led to a large increase in extrapolation number. Short exposure(within 3 hrs before or after) led to very variable results and both extrapolation number and D_0 could be increased or decreased depending on the exact timing of the experiment. Recovery experiments following 24 hrs exposure showed that where a dose was split by a 3-4 hr interval,increased recovery could be demonstrated but a 1-2 hr. gap between doses did not lead to any increase.

Fig. I shows the survival and recovery potential of cells following 24hrs. exposure to isobutyl methyl xanthine



OXYGEN DEPRIVATION/ENERGY METABOLISM

Prolonged (24 hrs.) deprivation of oxygen of cells in tissue culture was known to reduce the survival curve shoulder at the beginning of the project but details of O_2 levels below which the effect occured were unknown and no limits were available as to possible mechanisms. Work in the last five years confirmed the effect and also uncovered what may be a possible biochemical mechanism. Since lactate is the major end product of anaerobic glucose metabolism this was added to cultures and it produced a clear reduction in the extrapolation number of the survival curve, similar in magnitude to that observed with oxygen deprivation. The effect occured at levels obtained in tissue cultures and had no effect on growth rate. As an attempt to clarify the mechanism, other substances which reduce energy production but not through oxygen deprivation or lactate accumulation, were examined. These included non utilisable Glucose analogues and inhibitors of glycolysis. These also reduced cell survival following irradiation. Taken in conjunction with published information on the effects of ATP and respiratory and respiratory uncouplers on radiation response the results suggest an involvement of energy metabolism in the expression of radiation damage. The picture is complicated considerably by the effects of lactate on split dose recovery. A reduction in survival curve shoulder is normally associated with a reduction in recovery but prolonged lactate treatment increased the recovery of cells. The results were confirmed with secondary survival curves. Where the primary curve shoulder was reduced by lactate but the secondary curve shoulder was increased by lactate. This occurred at initial doses of 6, 7.5, 10, and 12.5 Grey. The figure shows the primary and secondary curves where the initial dose for the secondary curve was 10 Gy.



An understanding of this complicated effect (which may involve feedback effects resulting from energy demand) could help the understanding of the factors which lead to the production of a shouldered survival curve.

ETHANOL

Studies with ethanol resulted from it's use as a solvent for Vit. A work. It was found to have a radiobiological effect resulting in an increase in D_o when added between 3 and 6 hrs. prior to irradiation. No effect is seen if ethanol is added within 3 hrs. of irradiation. This effect may result from a stimulation of cellular energy metabolism since it occurred to a lesser degree in flasks which were simply given fresh medium within the 3 - 6 hr. period prior to irradiation.

GENERAL CONCLUSIONS

The results confirm the initial idea that useful radiobiological modifiers can be found among the relatively non-toxic physiological substances. The project has been successful in identifying or adding to the literature clear effects associated with Vitamin A, Vitamin C, prolonged oxygen deprivation, Lactate(Sodium and Calcium Salts), Ethanol and cAMP elevating substances (prostaglandins and methyl xanthine). From the detailed results in this report it can be seen that the cellular metabolic state has a considerable influence on the expression of radiation damage. Work with Oxygen deprivation, lactate and Glucose metabolism suggests an effect which could influence the interpretation of oxygen effects and tumour radiobiology as well as pointing to the importance of culture conditions in radiobiological experiments based on tissue culture systems. The work with Vitamin A and cAMP could suggest membrane involvement in radiobiological response but since both substances have documented effects on the cell nucleus, it is not possible to draw firm conclusions.

RESULTS OF PROJECT: NO. 2

HEAD OF PROJECT AND SCIENTIFIC STAFF: I.A. Kinsella

TITLE: Quantitative methods for fitting dose-effect curves and their extrapolation to low doses.

The initial phase of this project involved the writing of a computer programme suite to provide Maximum Likelihood estimates of the parameters of commonly used radiobiological models. The goodness of fit of these models was quantified and an index which could be used to discriminate between alternative competing models is computed. The method of estimation, due to the non-linearity of the parameters, used a search algorithm and is suitable for implementation of small to medium size main frames with double precision FORTRAN 4.

The second phase of the project involved explanation of the techniques available to aid optimum radiation dose choice to maximize the information available relating to the parameters. A computer programme was written which allows the user examine the effect of varying the spacings of the radiation doses across the viabli experimental range and, in general, the results indicate a plateau effect occurring quite rapidly. Information on costs could be used to optimize in the budgetary sense the experimentation regime and investigate alternative dose spacings.

The problem of extrapolation to low dose regions proved difficult to solve due to the inherent non-linearity of the parameters. An approximate solution involving the use of parameter confidence bounds with systematic evaluation of the survival modes was tried.

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Contractant de la Commission : Université Libre de Bruxelles

N° du contrat : 224-76-1 BIO B

Chef du groupe de recherche : Miroslav RADMAN

Thème général du contrat : Genetic Effects of Radiations and Chemical Carcinogens : Molecular Mechanisms common to DNA Repair, Recombination, Mutagenesis and Viral Induction in Bacteria and Mammalian Cells.

Titre du projet n°1 : Enzymology and Genetics of Mutagenesis and Recombinogenesis in E.coli and Mammalian Cells.

Chef du projet et collaborateurs scientifiques : M. Radman, S. Boiteux, F. Bourguignon-Van Horen, A. Brandenburger, C. Dambly, C. Dohet, O.P. Doubleday, P. Jeggo, A.R. Kinsella, P. Lecomte, Z. Toman, N. Tomilin.

This project, one of four internationally coordinated projects in the GERCC programme, was directed toward the study of (1) the chemical nature of the radiation and chemically-induced DNA lesions responsible for the induction of mutagenic repair and/or acting as targets for mutation fixation, (2) the accuracy and extent of DNA replication in vivo and in vitro upon radiation and chemically modified DNA templates (i.e. the mechanisms of mutagenesis at the molecular level), (3) the enzymology and genetics of the various systems responsible for the remarkable fidelity of DNA replication in bacteria and mammalian cells, (4) the mechanisms of the various modes of DNA recombination (rearrangements) and their relationship to mutagenic and carcinogenic cellular events and (5) the involvement of DNA alterations in the process of carcinogenesis.

The results of the last few years of our work in these five areas of research can be summarized as follows : (1) Our studies of UV and γ-ray induced mutagenesis, involving DNA sequencing of numerous mutations in phage M13 of E.coli suggest that a major portion of radiation induced base substitutions are due to "untargeted" mutagenesis resulting from the SOS induction of the host cell (ref 28). Lesions that arrest DNA synthesis and miscoding and non-coding lesions in UV irradiated poly(dC) homopolymers have been identified (ref 33). The treatment of homopolymers poly(dC) and poly(dA) with the active metabolites (epoxide and aldehyde) of the vinyl chloride monomer cause high frequencies of specific misincorporations during DNA synthesis (refs 27, 29). In vivo experiments have demonstrated a direct (SOS-independent) mutagenic potential of vinyl chloride metabolites. The exact nature of the SOS-inducing signal has not yet been established (collaborations with Drs. N. Godson, Yale University USA ; B.W. Glickman and L. van Sluis, University of Leiden Netherlands ; A. Barbin and H. Bartsch, W.H.O., IARC Lyon France).

(2) A fundamental experiment with single-stranded ØX174 phage showed that in vivo mutagenesis by UV light, and probably by any mutagen causing non-coding DNA lesions, requires SOS induction which allows erroneous copying of the DNA lesions i.e. "bypass" synthesis (ref 5). The inducible nature and induction kinetics have also been determined (ref 1). In an attempt to produce UV mutagenesis in vitro, an important role of the 3' to 5' exo-nuclease "proof reading" has been elucidated (ref 11). While a transient inactivation of this "editing" function of bacterial DNA polymerases may be necessary for the mutagenic "bypass" synthesis, it appears that the loss of exonuclease is not sufficient for mutagenesis which may require also a loss of base selection capacity (ref 30). An exonuclease with some "proof reading" properties has been identified in calf spleen (refs 13, 15, 20). Mutagenic metals Be^{++} and Mu^{++}, which affect base selection by DNA polymerases, cause increased in vitro mutagenesis on irradiated templates (refs 15, 34) (collaboration with Dr. S. Spadari, University of Pavia, Italy)

(3) Major progress towards an understanding of the molecular mechanisms responsible for the high fidelity of DNA replication (10^{-10} errors per nucleotide replication) has been achieved. Our research has showed that replicative and post-replicative "proof reading" mechanisms operate in conjunction with DNA replication in vivo.

Replicative proofreading consists of polymerase base selection and renewal of incorrectly incorporated nucleotides by a polymerase-associated 3' to 5' exonuclease activity (ref 20). The fidelity of the replicative eukaryotic DNA polymerase α is inversely proportional to the speed of replication (ref 31) which may explain the slow rate of replication in mammalian cells.

Postreplicative proofreading involves repair of replication errors (mis-matched base pairs in the newly synthesized DNA) by mutH, mutL, mutS and mutU gene products of E.coli (ref 22). The chemical basis for the enzymatic discrimination between the parental and newly synthesized DNA strands appears to be the methylation of adenine in 5' GATC 3' sequences (ref 21) : the newly synthesized strands are transitorily undermethylated.

This conclusion is derived from an analysis of progeny phages obtained from various E.coli strains transfected with heteroduplexes constructed in vitro from methylated and unmethylated phage λ DNA and containing mismatches at known sites (refs 21,31). Methylation-instructed postreplicative mismatch correction accounts for three to four orders of magnitude of replicational fidelity (ref 22) (collaboration with Drs. M. Meselson and R. Wagner (Harvard University, USA) and B.W. Glickman, University of Leiden, The Netherlands)

(4) Homologous recombination, both spontaneous and radiation-induced, seems to be a highly accurate process generating few if any mutations in E.coli (refs 12, 35). Non homologous recombination generates altered DNA sequences and is therefore mutagenic (translocations, inversions, deletions). Non-homologous recombination is the major source of genetic instability in mammalian cells. We have provided initial evidence (based upon the selective inhibition of chromosomal aberrations by antipain) that chromosomal aberrations induced by a chemical (MNNG) may be the result of an inducible process, hence subject to prevention(ref 23).The formation of X-ray induced chromosomal aberrations does not appear to be inhibited by antipain.

(5) The observation that a tumor promoter (TPA) induced SCEs which were inhibited by antipromoters (ref 18), led us to propose that promoter-induced or carcinogen-induced chromosomal rearrangements may be involved as one rate-limiting step in carcinogenesis (ref 24).

Relevance to radioprotection.

The demonstrated inducibility of radiation mutagenesis in bacteria (ref 1) and the possibility that mutagenesis and chromosomal aberrations may also be caused by inducible processes in mammalian cells (refs 23, 25) suggests that radiation-induced genetic alterations may be preventable. The fact that a protease inhibitor, antipain, inhibits : (1) UV mutagenesis in E.coli,(2) chromosomal aberrations in hamster cells,(3) in vitro transformation of X-ray irradiated or chemically treated mouse cells and (4) in vivo carcinogenesis on mouse skin may indicate that the prevention of genetic damage to man may become subject to prevention in a manner formally analogous to prevention of infective diseases. We can estimate that chromosomal aberrations, both spontaneous and radiation-induced, present several orders of magnitude higher genetic risk than does base change mutagenesis. As an initial application of the results of our work, we have constructed an E.coli tester strain, which simultaneously detects all genetic effects of DNA lesions : SOS induction, mutagenesis and recombination (refs 26, 32). Compared with UV light and several chemical mutagens, ionizing radiation (γ rays) is a much more potent recombinogen and SOS inducer than mutagen. Our tester strain is highly sensitive and can be adapted as a biological dosimeter for monitoring environments where people are working at high radiation risks. (see contract 156-76-1 BIO B, project 3,1c).

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Contraente della Commissione: Istituto di Genetica Biochimica ed Evoluzionistica del CNR, via S. Epifanio 14, 27100 Pavia, Italy

N° del contratto : 152-76-1 BIOI

Capo del gruppo di ricerca: Prof. Arturo Falaschi

Tema generale del contratto: Genetic effects of radiations and chemical carcinogens: molecular mechanisms common to DNA repair, recombination, mutagenesis and viral induction in bacteria and mammalian cells.

Titolo del progetto : Enzymes of DNA repair in human cells

Capo progetto e collaboratori scientifici: A. Falaschi, G. Ciarrocchi, F. Nuzzo, M.A. Pedrini, S. Spadari, M. Stefanini, U. Bertazzoni (EURATOM official)

During the past five years (1976-1980) we have pursued the following goals:

1) Study of the properties of DNA enzymes presumably involved in DNA repair and/or replication in human cells; this basic work is essential to describe the molecules available.
2) Study of the functions of the enzymes acting on DNA in animal cells in the different aspects of DNA metabolism. Information about their most likely role can be achieved by determining their levels in different physiological conditions, studying their distribution within the different cell compartments, understanding their responsibilities in the reparative and replicative processes.
3) Study of the properties of the cells derived from patients affected by inherited diseases involving DNA repair. These can be considered as human mutants of DNA repair and the description of the alterations of their properties at the cellular and molecular levels could give unique insights into the most important aspects of the repair processes.
4) Reconstruction of the DNA repair processes in vitro. The study involves the isolation of subcellular systems capable of functioning as "DNA repair complexes". The characteriza-

tion of the systems is to be ascertained in their capacity to respond to radiation damage in the presence of cellular or nuclear extracts and/or with purified protein fractions.

The results we have achieved can be summarized as follows:

a) We have purified from animal cells the DNA polymerases α, β and γ, the DNase VI, the DNA-dependent ATPase, the DNA binding proteins and the terminal deoxynucleotidyl transferase. The characterization of these enzymes has been very important for the precise setting of specific assays, thus enabling the determination of the activities in tissue and cell extracts. We have described the distinctive properties and the requirements of such enzymes permitting a better evaluation of their functions and a better comparison with similar enzymes described in bacterial systems.

b) Understanding of the functional roles of animal DNA polymerases in DNA replication and repair. Our work on the variations of DNA polymerase levels in stimulated lymphocytes has pointed out for the first time a direct indication of an involvement of β-polymerase in the process of DNA repair. This finding has been further substantiated by the results obtained with neuronal rat nuclei where UV irradiation results in 7-10 fold stimulation of DNA repair synthesis attributable uniquely to DNA polymerase β. Concerning the DNA polymerase γ, we have demonstrated that the enzyme is localized in the mitochondria, is responsible for the replicative synthesis of mitochondrial DNA, and presents a common phylogeny in all vertebrate classes. Of particular interest are the results obtained by using aphidicolin, a specific inhibitor of the replicative DNA polymerase α, showing that DNA polymerase β is indeed responsible for the repair of nuclear DNA. We have demonstrated that the DNA-dependent ATPase purified from human cells is capable of enhancing the activity of α-polymerase on growing fork-like structure, and is stimulated by the presence of a DNA binding protein purified from calf thymus.

c) Study of DNA enzymes and DNA repair capacity in inherited diseases affecting DNA repair. We have found that the three DNA polymerases in fibroblasts of Xeroderma Pigmentosum, Fanconi's anemia, Bloom's syndrome, Ataxia telengiactasia, Progeria and Werner's syndrome are the same as in controls and suggested that the deficiency in DNA repair is not a DNA polymerase. In F.A. the capacity to repair UV damage was found to be lower than in normal subjects. Two sibs with XP group A were found to differ markedly in the residual repair synthesis showing in one case a mosaic distribution of autoradiography label. The capacity to repair UV in DNA damage has been found in 7 cases of Fanconi's anemia to be lower than in normal subjects.

d) Isolation of complex structures mimicking DNA replication and repair in human cells. The mechanism of DNA synthesis at the growing fork has been investigated in vitro with the aid of DNA-dependent ATPase and DNA binding protein allowing a 30 fold increase in the rate of DNA polymerase α on BK DNA molecules. The reconstruction of DNA repair processes in subcellular systems has been attempted in cellular lysates, isolated nuclei and isolated metaphase chromosomes. The systems have been partially characterized in their capacity to respond to radiation damage and to function as "DNA repair complexes". In particular, we have shown that the utilization of aphidicolin allows a rapid and simple evaluation of DNA-repair synthesis in damaged human cells. This repair assay, based on the complete inhibition of DNA replication due to α-polymerase is particularly promising for the detection of effects caused by mutagens in human cells.

The contribution of our group to the understanding of the functions of DNA polymerases α, β and γ and of DNA-dependent ATPase in DNA replication and repair has been very important and has largely exceeded our initial objectives. A great effort has been made in the characterization of human mutant cells, leading to a better definement of their physiology and defi-

ciency.

We think that the extreme importance of the molecules, biological processes and structures in the response to radiation damage completely justify our efforts of investigation and will continue to be fundamental in governing the problem of radioprotection of man.

The setting of a simple and precise method for the in vivo determination of DNA repair synthesis has been achieved.

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Contractor:

Centre National de la Recherche Scientifique,
Radiobiogie Cellulaire,
Laboratoire d'Enzymologie,
91190 GIF-SUR-YVETTE (France)

Contract number:

223-76-1-BIO-F

Heads of research teams:

R. DEVORET

General subject:

Genetic Effects of Radiations and Chemical Carcinogens: Molecular mechanisms common to DNA repair, recombination, mutagenesis and viral induction in bacteria and mammalian cells.

Title of project

DNA repair, recombination, mutagenesis and viral induction in Escherichia coli: genetic characterization of the recA gene and the role of RecA protein.

Head of research team and staff:

R. DEVORET, A. BAILONE, A. LEVINE, P. L. MOREAU AND M. FANICA

SUMMARY OF RESULTS OBTAINED FROM 1976 TO 1980

A. RELEVANCE TO RADIOPROTECTION

As radiation biologists, we are aware that the use and development of nuclear energy will be made safer if any possible radiation damage can be prevented. We feel it our duty to provide tools that will ensure better biological protection against radiations. Efficient protection against radiations depends on the recognition of the following radiobiological axioms:

1- **DNA is one of the most important target of radiations.** DNA is the hereditary material of all living cells. Athough X rays, gamma rays and other radiations hit all the components of cells in a random manner, the genetic material is the most radiation-sensitive cellular target. DNA constitutes a minor fraction of the chemical components of a cell, but direct or indirect damage to it has great impact on the cell's future, whereas damage to proteins and other cellular components has much less effect. That is because DNA is the memory of the cell. DNA replicates to beget DNA, and so errors in DNA are transmitted from cell generation to

cell generation.

2- **It is impossible to prevent every radiation damage.** A flow of radiations reaches the earth. All living cells are exposed to a natural background of radiations. In principle, there is no threshold for the effects of ionizing radiations, nevertheless life has evolved successfully on earth. Even though DNA is relatively protected from radiation damage by many cell constituents, its stability is mainly maintained not by shielding mechanisms but by processes that restore the integrity of the damaged structure. Recent progress in our knowledge of repair processes has been spectacular whereas that concerning biological protection has been poor in spite of numerous past efforts.

3- **Radiation damage can be efficiently repaired.** At low doses, most of the damage can be erased by the action of a variety of repair processes. To cope with the deleterious effects of radiations, Nature has invented a variety of repair processes that restore the damaged biological structures.

4- **Repair of radiation damage cannot be dissociated from repair of damage induced by chemicals.** A recent scientific breakthrough is the discovery that some natural antibiotics and many metabolized chemicals can damage DNA in a way similar to X-rays whereas some other of these compounds produce DNA lesions that are repaired by the very repair system that excise pyrimidine dimers from UV-damaged DNA. More generally, normal metabolism in man leads to the production of a variety of short-lived radicals that damage DNA. Recently accumulated knowledge has provided a wealth of data that underline the fact that radiations are not unique in their way of damaging DNA. The chemistry of how primary DNA lesions are produced has gained much from the study of chemical radicals formed in the course of metabolization.

5- **All repair processes known to occur in man have been identified first in bacteria.** Although the ultimate aim of radiation biologists is to heal DNA damage occurring in man's cells, bacteria still provide an invaluable tool to molecular radiation biology. For instance, it has been recently found in mammalian cells a process similar to inducible error-prone repair observed in bacteria.

Not only was our work based upon the above-mentioned axioms but it contributed to the formulation of the last 3 of them.

B. SUMMARY OF RESULTS OBTAINED

1- **RecA protein, keystone of cellular radioresistance.** We now know that RecA protein, induced by DNA damage, will constitute up to 10 %

of the total of cellular proteins (Paoletti, pers. comm.; Moreau et al., 1981). There is no other cellular protein induced by any cell treatment to such an extent. Cellular radioresistance (cell survival) to DNA damage produced by X-rays, UV light and chemicals is roughly proportional to the cellular concentration of RecA protein. Cellular radioresistance can be enhanced if the normal level of RecA protein is artificially increased. RecA protein is important not only quantitatively but also qualitatively. It controls constitutive processes such as genetic recombination as well as processes induced by DNA damage such as inducible error-prone repair (SOS repair). We (Quillardet et al., unpublished) demonstrated that inducible error-prone repair is independent of the cell concentration in RecA protein. In contrast, LexA protein, the repressor of the recA gene, plays a major role. The LexA protein controls genes other than recA that regulate repair and the fidelity of DNA replication such as umuC. The type of repair promoted by plasmid genes conferred by plasmid R46 and its derivative, pKM101, looks like but is different from SOS repair as we demonstrated it. Repair promoted by pKM101 escapes the control of LexA protein.

2- **Mapping and complementation of recA mutations.** We found that the recA gene can be divided into two parts according to the phenotypes of mutations called for that reason lexB and recA proper. Such mutations may reveal the existence of two main domains in RecA protein. The recA domain may direct the constitutive functions of RecA protein (binding to DNA, control of exonuclease V) whereas the lexB domain may be more specifically involved in inducible functions (proteolytic action on repressors). Radioresistance can be restored in recA$^-$ mutants by complementation of two altered non-functional RecA proteins.

3- **RecA-protein controls prophage induction.**

Roberts et al. discovered that RecA protein inactivates prophage repressors by cleavage. RecA protein appears to be the "inducer" of dormant viruses postulated by Jacob and Monod.

We observed that there is no correlation between the kinetics of λ repressor cleavage and the kinetics of accumulation of RecA protein. Since Little and associates found that RecA protein cleaves LexA protein, the repressor of RecA protein, it has been widely surmised that induction of RecA protein was required for prophage induction to occur. We demonstrated that full induction of RecA protein is not required either for prophage λ or for prophage 80.

A mutation in the recA gene, lexB30, that we isolated has little effect on recombination but renders prophage λ non-inducible. We observed that prophage 80 can be induced in a lexB30 lysogen. This mutation may

affect the binding affinity of RecA protein to repressors but not necessarily RecA protease activity.

4- **Initiation of DNA replication and prophage induction.** We have recently found that a portion encompassing the origin of the F sex factor controls indirect induction of prophage λ (Devoret, Couturier and von Meyenburg, to be published). Following DNA damage, an essential region near the origin of replication regulates DNA replication and repair.

5- **Tests for potential carcinogens.**

We have shown that prophage induction can be used as an efficient test to detect physical and potential chemical carcinogens. Our test is now being used by numerous public institutions as well as pharmaceutical companies.

C. **CONCLUSIONS.**

Five years ago, we proposed a project concerning the study of the recA gene and of the protein coded by this gene. The results obtained by a few international groups, including ours, on the cellular role of RecA protein have more than fulfilled the expectations. RecA protein is unique. It associates the activities of a protease with those of a DNA binding protein. RecA protein plays a unique role in the cell. It controls numerous basic cellular functions. The knowledge of its involvement in the mechanisms of the various repair processes can provide a valuable goal within the frame of a programme aimed at contributing to ensure radiation protection of mankind.

What is the mechanism whereby each radiation or chemical affects DNA? How are DNA lesions repaired? Only basic knowledge can breed real safety.

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Contractor: Department of Radiation Genetics and Chemical Mutagenesis (C.N.R.)

Contract No. 150-76-1 BIOI

Head of Research Team: Prof. Dr. G. Olivieri

General subject of Contract: Studies on induced chromosome aberrations and chromosome structure.

Project n.1: Studies on the factors affecting the pattern of rejoining (symmetric or asymmetric) in the formation of chromosomal exchanges

Head of Project: G. Olivieri

The bulk of the work done in the past four years can be summarized as follows:

1) We developed and defined the "diplochromosome test" (2,3):

Experiments have been performed in order to study the spontaneous appearance of cells with diplochromosomes (endoreduplicated cells) in Chinese Hamster cultures. Endoreduplicated cells are present in sub-cultures (sub-M-cultures) prepared by trypsinising cultures (M cultures) in the plateau phase of growth. Two conditions are particularly important for the appearance of endoreduplicated cells: a change of medium 3 days before trypsinisation and the growth of the M-cultures in a closed atmosphere from that moment on. The maximum frequency of endoreduplicated cells occurs when the M-cultures are grown in bottles that have been stoppered also during the 6-9 days prior to the change of medium. In these conditions the frequency of endoreduplicated cells found in the sub-M-cultures 26-30 hours after trypsinisation varies around 8%. In the crowed M-cultures, which grow in closed atmospheres, the change of medium is followed by an unbalanced cell growth that leads to a greater protein content per cell.

Endoreduplicated cells perform the first DNA synthesis before trypsinisation and the second after it; therefore these cells were blocked or slowed down at the moment of trypsinisation in a stage of the cycle with 4C of DNA. This stage is probably comparable to a G_1 stage that follows a first cell cycle that has concluded without a normal mitosis.

2) The variation of symmetrical/asymmetrical ratio was analyzed in relation to:

a) cell cycle (4,14,17):

The relative frequencies of X-ray-induced symmetrical and asymmetrical chromatid interchanges were analysed as a function of the cell cycle in somatic cells of Drosophila melanogaster.

In exchanges between autosomes, during the S phase, a clear prevalence of asymmetrical rejoining was observed. Later, this prevalence became reduced as the cell approached mitosis. On the other hand, in the exchanges between X-chromosomes in the females there were no significant variations in the type of rejoining through the cell cycle, there being a slight but consistent preference for symmetrical rejoining.

The symmetry of radiation-induced chromatid exchanges was studied in relation to the cell cycle in Chinese hamster cells in vivo and in vitro. Both in vivo and in vitro, the ratio between symmetrical and asymmetrical chromatid exchanges was about 1 to 1 during G_2 and S phase of the cell cycle.

We studied the variation of the symmetrical/asymmetrical ratio in chromatid interchanges during the various phases of the cell cycle of human lymphocytes in vitro. In the cells irradiated in S phase (labelled) there was a significant prevalence of asymmetrical exchanges, whereas in the cells in G_2 phase (unlabelled) there was no such prevalence.

b) type of cell treated (4,10,14,17): chinese hamster cells in vivo and in vitro, human lymphocytes, spermatocytes I and somatic cells of D.melanogaster we studied.

c) type of mutagen treatment (Mut. Res. 33: 201):

Third instar larvae of the Oregon R stock were treated for 25 or 30 min with vapour of methyl methanesulphonate (MMS). At various times after the treatment (4,8 and 12h), microscope specimens of the nerve ganglia were prepared. At all the times of fixation, only aberrations of a chromatid type were found, but with different frequencies in the two sexes. The females were about 3 times more sensitive than the males to MMS.

An analysis of the distribution of the breaks between and within chromosomes showed that they were not localized at random but were clustered in the heterochromatic centromere of the X chromosome and the autosomes. The Y chromosome, although entirely heterochromatic, on the other hand, was highly resistant to MMS. However, this

phenomenon had only a very weak effect upon the variation with sex of MMS-induced chromosome damage.

Among the aberrations induced, although interchanges were normally present, neither intra-exchanges nor triradials were found. In the interchanges, there was a greater tendency for the homologous chromosomes to be involved, and these rejoined symmetrically.

d) type of genetic constitution (24):

Repair- and recombination-defective mutations at two loci (mei-9 and mei-41) of Drosophila melanogaster have been examined for their effects on the induction of chromosome aberrations by x-rays and the formation of sister chromatid exchanges (SCEs). Irradiation of larval neuroblast cells during the S phase with x-rays showed that mutants at both of these loci are about 10 times more sensitive than wild type to the induction of chromosome aberrations. The pattern of induced aberrations was characteristic for each mutant locus: in cells bearing mei-9 mutations most breaks were chromatid deletions, whereas in the presence of mei-41 mutations similar frequencies of chromatid and isochromatid deletions were observed. Furthermore, chromatid interchanges could not be induced in cells carrying mei-9 alleles; therefore these mutations define a step necessary for chromatid rejoining. mei-41 alleles also define a function involved in the formation of chromatid interchanges; total exchanges were less frequent than expected from nonmutant controls; and the proportion of exchanges arising by symmetrical rejoining was markedly reduced. These data indicate that chromatid and isochromatid deletions have different molecular steps in their formation, and that different molecular mechanisms are also involved in the symmetrical and unsymmetrical rejoining in chromatid interchanges.

Taken as a whole our results indicate that, although physical proximity of chromosomes is an essential prerequisite for the formation of exchanges, the type of rejoining is determined at molecular level.

3) Important conclusions were also drawn on studying sister chromatid exchanges in Drosophila melanogaster (20,23,24,25):

Neural ganglia of wild type third-instar larvae of Drosophila melanogaster were in-

ubated for 13 hours at various concentrations of BUdR (1,3,9,27 µg/ml). Metaphases were collected with colchicine, stained with Hoechst 33258, and scored under a fluorescence microscope. Metaphases in which the sister chromatids were clearly differentiated were scored for the presence of sister-chromatid exchanges (SCEs). At the lowest concentration of BUdR (1 µg/ml), no SCEs were observed in either male or female neuroblasts. The SCEs were found at the higher concentrations of BUdR (3,9 and 27 µg/ml) and with a greater frequency in females than in males. Therefore SCEs are not a spontaneous phenomenon in Drosophila melanogaster, but are induced by BUdR incorporated in the DNA. A striking nonrandomness was found in the distribution of SCEs along the chromosomes. More than a third of the SCEs were clustered in the junctions between euchromatin and heterochromatin. The remaining SCEs were preferentially localized within the heterochromatic regions of the X chromosome and the autosomes and primarily on the entirely heterochromatic Y Chromosome. -In order to find an alternative way of measuring the frequency of SCEs in Drosophila neuroblasts, the occurrence of double dicentric rings was studied in two stocks carrying monocentric ring-X chromosomes. One ring chromosome, C(1)TR 94-2, shows a rate of dicentric ring formation corresponding to the frequency of SCEs observed in the BUdR-labelled rod chromosomes. The other ring studied, R(1)2, exhibits a frequency of SCEs higher than that observed with both C(1)TR 94-2 and rod chromosomes.

Taken as whole these results indicate a lack of spontaneous SCEs in neuroblast metaphases of Drosophila melanogaster. In addition some insight was gained into the relationships between SCEs, chromosome aberrations and DNA repair.

Numbers in brakets are referred to the publications.

Project n.2: Study of factors (genetic and environmental) modifying the frequency and type of chromosomal aberrations

Head of Project: M. Gatti

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a) The effect of the state of nutrition on radiosensitivity in Drosophila melanogaster was studied (8):

Body-weight has been shown to influence the final expression of genetic damage by X-rays in Drosophila melanogaster. If larvae of Drosophila were raised up to the third instar in media containing different amounts of the same nutrient and in different conditions of crowding a positive correlations was observed between body-weight and frequency of chromosome aberrations induced by a given dose of X-rays in the somatic cells of their nerve ganglia. This effect, present in both sexes, is most plausibly attributed to a different capacity of big and small larvae for repairing radiation damage.

b) The radiosentivity of Drosophila somatic chromosomes irradiated in C-metaphase was analyzed in comparison with that of interphase chromosomes (19):

Neural ganglia of Drosophila melanogaster were placed in saline containing colchicine. After two hours, they were irradiated and then samples were fixed at 5,15,25, 35 minutes from the beginning of irradiation. The results obtained show the aberration level increases with time subsequent to fixing. This increase takes place first for chromatid deletions and then for isochromatid deletions and chromatid exchanges. Gaps and subchromatid exchanges do not, on the contrary, show any increase with time.

c) The mutagenic action of caffeine in Drosophila melanogaster was investigated(22):

Nerve ganglia of third-instar larvae were treated with various doses of caffeine ($5x10^{-4}$, 10^{-3}, $5x10^{-3}$, 10^{-2} and $2x10^{-2}$M) for 2 h at 25±1°C. The ganglia were fixed at set time intervals after treatment so that the effect of caffeine in different stages of the cell cycle could be observed. Chromatid aberrations were indu

ced only when the caffeine was administered in G_2 or approaching mitosis. No aberrations were observed after treatment in S or early G_2. In relation to the different doses administered, a threshold effect was evidenced, the number of aberrations increasing in a marked way at doses exceeding 5×10^{-3}M. These data indicate that the effect observed in Drosophila melanogaster is similar to that described by Kihlman in animals and plants treated with caffeine at temperature below 30°C.

d) The relations between premutational damage, DNA repair processes, chromosome aberrations and SCEs were in part clarified (16,19,22,23,24,25):

Eight X-linked recombination-defective meiotic mutants (representing five loci) and 12 X-linked mutagen-sensitive mutants (representing seven loci) of Drosophila melanogaster have been examined cytologically in neuroblast metaphases for their effects on the frequencies and types of spontaneous chromosome aberrations. Twelve mutants, representing five loci, significantly increase the frequency of chromosomal aberrations. The mutants at these five loci, however, differ markedly both in the types of aberrations produced and the localization of their effects along the chromosome. According to these criteria, the mutants can be assigned to four groups: (i) mutants producing almost exclusively chromatid breaks in both euchromatin and heterochromatin; (ii) mutants producing chromatid and isochromatid breaks in both euchromatin and heterochromatin; (iii) mutants producing chromatid and isochromatid breaks primarily in euchromatin; and (iv) mutants producing chromatid and isochromatid breaks clustered in the heterochromatin.

Neuroblast cells of larvae bearing mei-9 and mei-41 alleles were also treated for 13 hr with 5-bromodeoxyuridine at 9 µg/ml in order to differentiate sister chromatids for the scoring of SCEs. Whereas mei-41 had a normal level of SCEs, mei-9 exhibited a frequency of SCEs that was about 70% that the control. Because both mei-9 and mei-41 mutations result in defective meiotic recombination, these data suggest that they define steps shared by symmetrical interchange formation and meiotic recombination that do not participate in the formation of most SCEs. These studies are been currently-continued and expanded in our laboratory (24).

Numbers in brakets are referred to the publications.

Project n.3: Study of the transmission of chromosomal aberrations

Head of Project: A. De Marco

The relative frequencies of X ray induced chromosome aberrations in Drosophila neuroblast cells, and in spermatocytes of females irradiated with 625R X-rays were cytologically compared (10).

The spermatocytes were more sensitive than the gangliar cells to radiation: the aberrations induced in the spermatocytes I were three times more frequent than those induced in the ganglia. The distribution of the aberrations was, however, similar. The most important variable element lies in our observing no symmetrical exchanges in the spermatocytes.

Now we intend to study how many and which aberrations are transmitted to the F_1 in the case of the germinal line and to subsequent cell generations in the case of the somatic line.

Numbers in brakets are referred to the publications.

Project n.4: Study of the relationships between structure of the chromosome and aberrations produced

Head of Project: M. Gatti

A series of experiments were carried out in order to obtain a better resolution in chromosome banding and understand the chemical basis of banding. These experiments were focused on Drosophila (1,5) and other diptera (12,21) and addressed to differentiate the heterochromatic regions.

A number of preliminary experiments have shown that the fluorescence pattern of Hoechst 33258, as opposed to that of quinacrine, varies with the concentration of dye. The metaphase chromosomes of Drosophila melanogaster, D.similans, D.virilis, D.texana, D.hydei and D.ezoana have therefore been stained with two concentrations of H. 33258 (0.05 and 0.5 ug/ml in phosphate buffer at pH7) and with a single concentration of quinacrine (0.5% in absolute alcohol). The three fluorescence patterns so obtained were shown to be somewhat different in some of the species and the coincide in others. All three stainings gave an excellent longitudinal differentiation of heterochromatin while euchromatin fluoresced homogeneously. -Living ganglion cells of the six species mentioned above were treated with quinacrine and H.33258. Quinacrine induced a generalized lengthening and swelling of the chromosomes and H. 33258 the decondensation of specific heterochromatic regions.- A correlation of the base composition of the satellite DNAs contained in the heterochromatin of the species studied with the relative fluorescence and decondensation patterns showed that: 1) the extremely fluorochrome bright areas and those decondensed are present only in species containing AT rich satellite DNA; 2) the opposite is not true since some AT-rich satellite DNAs are neither fluorochrome bright nor decondensed; 3) there is no good correspondence between Hoechst bright areas and the decondensed ones. -AT richness therefore appears to be a necessary but not sufficient condition both for bright fluorescence and decondensation. Some cytological evidence

suggests that similarly AT rich satellite DNA's respond differently in fluorescence and decondensation because they are bound to different chromosomal proteins. A combination of the results of fluorescence and decondensation revealed at least 14 types of heterochromatin; 4-7 of which are simultaneously present in the same species. Since closely related species (i.e. D. melanogaster and D. simulans; D. virilis and D.texana) show marked differences in the heterochromatic types they contain, it can be suggested that within the genus Drosophila qualitative variations of heterochromatin have played an important role in speciation.

The C- and N-banding patterns of Drosophila melanogaster, D.simulans, D.virilis, D.texana, D.ezoana and D.hydei were studied in comparison with quinacrine and Hoechst banding patterns. In all these Drosophila species the C bands correspond to the heterochromatin as revealed by the positive heteropycnosis in the prometaphase chromosomes. The N bands have the following characteristics: 1) they are always localized on the heterochromatin and generally do not correspond to the C bands; 2) they do not correspond to the nucleolar organizing regions; 3) they are inversely correlated with fluorescence, i.e., they correspond to regions which are scarcely, if at all, fluorescent after Hoechst 33258 or quinacrine staining; 4) they are localized both on regions containing AT rich satellite DNA and on those containing GC rich satellite DNA.

The banding mechanisms of Chromomycin and Olivomycin were also investigated in human and mouse chromosomes (18):

Human and mouse chromosomes, stained with either chromomycin A_3 or olivomycin, which bind preferentially to G C -rich DNA (where G is guanosine and C is cytosine), exhibit a Q or a reverse banding pattern, depending on the wavelength used for excitation. The two complementary banding patterns can be observed in the same metaphase simply by changing the combination of excitation filters. These data suggest, therefore, that in addition to base composition, other factors are involved in the production of chromosome banding by chromomycin A_3 and olivomycin.

Moreover several compounds affecting chromosome condensation were tested in vitro for their effects on Drosophila, human and Chinese hamster chromosomes (6,7,9,15). These studies have permitted us to reach some conclusions on the relationships between chromosome structure and distribution of induced aberration (11).

The distribution of chromosomal aberrations between and within chromosomes of male D.melanogaster somatic cells after treatment with UV has been analyzed. Distribution of the breaks between chromosomes was largely nonrandom since we found a higher aberration frequency than that expected on the Y chromosome. Moreover, within the chromosomes the aberrations are clustered in the pericentromeric heterochromatic regions. The above distribution compared with that of the breaks induced by X rays and methyl-methane-sulphonate (MMS) suggests that there are two classes of heterochromatin in D.melanogaster. The first class, which includes the heterochromatin of the X chromosome and of the autosomes, has a consistently greater sensitivity than euchromatin, independent of the mutagen used. This class probably has a base composition on the average not very different from that of euchromatin, and its "sensitivity" could be related to factors such as its greater compaction in interphase (Smith and Evans,1976) or to its peripheral location in the nucleus (Hsu,1975) or possibly to different repair mechanisms (Falk,1961).

The Y chromosome constitutes the second class of heterochromatin, which differs from the first in its exceptional AT richness which is related to its different sensitivity with respect to euchromatin and the other heterochromatic regions, depending on the type of mutagen used.

Numbers in brakets are referred to the publications.

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Vertragspartner der Kommission: Universität Göttingen
Institut für Humangenetik

Nr. des Vertrages: 206-76-7 BIO D

Leiter der Forschungsgruppe: Priv.Doz.Dr.I.Hansmann

Allgemeines Thema des Vertrages:

Cytogenetic studies in mammalian oogenesis and spermatogenesis on the induction of nondisjunction and structural chromosome anomalies, with special consideration of the genetic risk in the F_1 population.

Titel des Projektes Nr. 1:

Induction of non-disjunction in oogenesis.

Leiter des Projekts und wissenschaftliche Mitarbeiter: Dr.I.Hansmann
Dr.D.Janke
Dr.H.-D.Probeck

The epidemiological studies in human populations allow, so far, no definite answer to the question whether the incidence of Down's-Syndrome among new-borns may be increased by exposing mothers with X-rays before conception. Either X-rays are harmless in inducing non-disjunction under those circumstances applied or they potentially induce non-disjunction; their effects, however, in a certain population are not simply in just one direction.

In two earlier experiments it was shown that irradiation of female mice increased significantly the number of hyperploid ovulated oocytes.

We followed this question and studied three mammalian species (mouse, chinese hamster, Djungarian hamster) and analysed chromosomes from oocytes after the first meiotic division immediately

after ovulation and long before selection against chromosomally unbalanced gametes/embryos may occur. The experimental conditions of irradiation and handling of the animals were the same during the course of the experiments. We tried to answer the following questions:

1. Can non-disjunction be induced in mammalian oocytes and is the incidence dependend on the dose of X-rays?

2. Have we to consider species-specific effects?

3. Is the constitution of the female important for the sensitivity, e.g. does the age and the hormonal constitution interfere with radiosensitivity?

In all experiments females from the above mentioned species were pretreated with PMS (pregnant mares serum) and HCG (human chorionic gonadotrophin) 48 h later for synchronized ovulation. Irradiation was done 3 h after the HCG injection. This stage was proven earlier by us to be a very sensitive period for the induction of non-disjunction by chemical mutagens. Chromosomes were analysed after ovulation at the stage of metaphase II. Hypoploid metaphases were not included, because preparational artifacts can not be excluded. Oocytes were handled singly, so metaphases with more than the haploid chromosome set (e.g. n=21; n=12; n=29) can never be due to an artifact, but result from non-disjunction during first meiotic division.

In one experiment we used females from a mouse strain (NMRI) which shows a genetically determined genommutation (ovulation of some diploid oocytes spontaneously) and which can be stimulated to a hypergonadotrophic situation.

The females received 10 i.u. PMS and 10 i.u. HCG and were irradiated with various doses from 5 to 80 rad.

We did not observe any significant increase of hyperploid oocytes, neither considering the results from individual doses nor the summarized data (Table 1). No increase of hyperploidy with increasing dose was observed.

Table 1: Non-disjunction in NMRI-mouse oocytes after irradiation during the preovulatory phase

dose (rad)	no. of oocytes analysed	% of oocytes with 21 chromosomes
5	264	0.38
10	239	0.0
20	250	0.8
40	220	0.0
80	229	0.9
Total	1.202	0.42
control	501	0.20

After the same kind of treatment, however, the incidence of diploid ovulated oocytes increased with radiation dose. This strain ovulates spontaneously, as already mentioned, diploid oocytes. Incidence figures increased with irradiation dose: 2.5 % (control); 3.1 % (5 rad); 9.6 % (10 rad); 6.0 % (20 rad); 6.8 % (40 rad); 12.7 % (80 rad).

Recently it was shown that aging mouse oocytes are more sensitive for radiation-induced non-disjunction than those from younger females (Uchida, I.A. and C.P.V.Freeman, Nature (Lond.) 265 (1977) 186-187).

We studied chinese hamster females (Cricetulus griseus),to see whether the higher sensitivity in aging females is a more general phenomenon in mammals or whether it is restricted to mice only. Females at various ages received 3 i.u. PMS and 2 i.u. HCG for stimulated ovulation and were irradiated 3 h

after HCG with 20 rad (Table 2):

Table 2: Non-disjunction in chinese hamster oocytes after irradiation during the preovulatory phase

dose (rad)	age of females (weeks)	no. of oocytes analysed	% of oocytes with n=12
0	8-20	129	0.0
20	8-20	325	0.0
	36-48	63	0.0
	≥56	99	3.0

No significant increase was detected at ages of 8-20 oder 36-48 weeks compared to nonirradiated females. The incidence of hyperploidy in the irradiated higher age group was significantly increased.

Djungarian hamster females (Phodopus sungorus) ovulate oocytes after hormonal stimulation which are hyperploid. The incidence of hyperploidy is dependend on the doses of gonadotrophins injected. Two experiments were performed: One, in which the females were stimulated with low doses of hormones (3 i.u. PMS and 2 i.u. HCG) to have a lower spontaneous incidence of non-disjunction and a second one, during which females were stimulated with higher doses (10 i.u. PMS and 10 i.u. HCG) to induce a high incidence of genommutations in non-irradiated females. A group of females received 20 rad 3 h after HCG in each experiment (Table 3):

Table 3: Genommutations in Djungarian hamster females after hormonal stimulation and irradiation

dose of hormones (i.u.PMS/i.u.HCG)	dose (rad)	no.of oocytes analysed	% of oocytes with a genommutation *)
3/2	0	184	2.7
3/2	20	363	0.7
10/10	0	278	17.2
10/10	20	513	5.1

*) both types: hyperploidy and diploidy

In both experiments, low and high hormone group, is the incidence of genommutations after exposure to 20 rad lower than in the oocytes from the non-exposed females. The decrease even in the incidence of hyperploidy only, is statistically signifikant in the 10/10-hormone group: 8.2 % in non-irradiated and 4.3 % after exposure to 20 rad.

The observed "protecting" effect of X-rays on the incidence of genommutations cannot be explained by cellkilling, because the number of ovulated oocytes was not reduced. The mechanism must be different. Analysis for structural abnormalities (breaks, fragments, deletions) in the same oocytes demonstrated, however, that the X-rays applied to the females were biologically active within the oocytes: No structural anomaly was detected in non-irradiated oocytes, the incidence increased to 7.2 % (3 i.u. PMS/2 i.u. HCG) and 7.7 % (10 i.u. HCG) respectively in the 20 rad-groups.

From our data and the few published studies in the literature we conclude that X-rays do interfere with chromosome segregation during meiosis, but differently. The direction of interference seems to depend on the constitution (e.g. strain, age,

hormonal constitution) of the females; X-rays may:

1. induce non-disjunction in sensitive strains, species, subpopulations

2. not induce non-disjunction in insensitive/resistant females

3. decrease the incidence of non-disjunction in oocytes from those females, which do have a high "spontaneous" risk for aneuploidy, e.g. females in a hypergonadotrophic situation.

This three possible ways of interference may explain, why in a given population the total incidence of aneuploidy must not necessarily increase after exposure to X-rays. The frequency of genommutations depend then on the relative size of the subpopulations.

Titel des Projekts Nr. 2:

Stage sensitivity in oogenesis.

Leiter des Projekts und wissenschaftliche Mitarbeiter: Dr.I.Hansmann
Dr.D.Janke
Dr.H.-D.Probeck

The aim of this project was to investigate the sensitivity of different stages during mouse oogenesis for the induction of chromosome anomalies by X-rays.

Females were irradiated at different ages (1, 3, 6 and 8-12 weeks) with different X-ray doses and oocytes prepared from these irradiated females at the age of 8-12 weeks. Chromosome analysis was either done on metaphase I-oocytes or after ovulation on metaphase II-chromosomes. The results so far obtained indicate a high sensitivity for radiation-induced translocations during the final phase of oogenesis. The effectiveness of X-rays to produce structural anomalies which are detectable later in mature oocytes, is lower in early stages of dictyotene (e.g. 3 weeks). This may be due to an concomitantly high sensitivity for cellkilling (e.g. in oocytes from 1 week-old females) resulting insterility at even low doses.

In recent experiments we studied the influence of coffein on the expression of radiation-induced translocations and injected coffein before an after irradiation. Chromosome analysis at metaphase I is still in progress and the final results will be reported.

In addition to these studies we are able to compare our results on radiation-induced structural anomalies from different species. The experimental conditions, such as handling of the animals, conditions of X-ray exposure, method of chromosome preparation and analysis as well as investigators, are identical. This comparison therefore is valid to estimate the radiosensitivity in oocytes from 3 different species. Data are available after irradiation with 20 rad.

Table 1: Structural chromosome anomalies in oocytes from 3 different species after exposure to 20 rad.

species	no. of oocytes analysed	% of oocytes with structural anomaly
mouse-NMRI	264	3.8
Chinese hamster	325	4.6
Djungarian hamster	139	7.2
	235[1)]	7.7

[1)] hormonal synchronization with 10 i.u.PMS/10 i.u.HCG

The hormonal pretreatment - low or high doses - did not have an influence on the expression of radiation-induced structural anomalies. This conclusion can be drawn comparing the results from the Djungarian hamster. Oocytes from the Djungarian hamster do express, however, significantly ($P < 0.05$) more structural anomalies (breaks, fragments, translocations) than oocytes from NMRI-mice. The difference can be due to a different timing of progressing through meiosis after germinal vesicle break-down, different repair-capacities or different sensitivity to hormonal induced processes (activation of enzyme systems) in different species.

An interesting finding in this context is our observation on an increasing expression of radiation-induced chromosome breakage with increasing age in chinese hamster oocytes.

Table 2: Structural anomalies in oocytes from chinese hamsters after exposure to 20 rad.

age (weeks)	no. of analysed oocytes	% of oocytes with structural anomalies
8-20	325	4.6
36-48	63	7.9
$\geq$ 56	99	13.1

The higher incidence in the agegroup of $\geq$ 56 weeks-old females is statistically significant ($P < 0.05$). The conclusion from the study is, that with increasing age of the females not only the risk for radiation-induced aneuploidy increase, but that also more chromosome breaks are expressed in aging ovulated oocytes. The reason for this is unclear, but the finding may be explained either by a different sensitivity per se, a decreasing repair-capacity or by a different progression through meiosis after germinal vesicle break-down.

Our findings of a different sensitivity at different stages of oogenesis, at different ages of the females as well as a different sensitivity between 3 mammalian species are important for the problem of risk estimation as well as the question of extrapolating animal data to man.

Titel des Projekts Nr. 3:

Risk for chromosome anomalies in the F_1-population after maternal and paternal exposure to X-rays.

Leiter des Projekts und wissenschaftliche Mitarbeiter: Dr.I.Hansmann
Dr.D.Janke
Dr.H.-D.Probeck

The aim of project 3 was to study cytogenetically the radiation effects in maternal and paternal germ cells after exposure to 0,20 and 200 rad. Special attention was given in these studies to the risk-estimation for chromosome anomalies in the F_1-generation after parental exposure to X-rays. The aim was to base the risk-estimation on results, obtained with modern cytogenetic techniques, i.e. chromosome banding techniques for the assessment of small structural chromosome abnormalities. After a pilot study at the beginning of the project we were able to standardize a G-banding technique. The results obtained with this method demonstrate, that nearly all structural abnormalities in 9.5 day-old F_1-embryos were only detectable after G-banding followed by karyotyping of mouse chromosomes. No radiation-induced structural abnormality resulted in very large or very small marker chromosomes or in chromosomes with an altered centromerposition (meta- or submeta centric). These altered chromosomes are detectable by conventional techniques.

In addition we were able to distinguish between balanced and unbalanced translocations, which can never be done by standard staining methods. Another advantage of this technique proved to be the possibility to corroborate trisomies, or more important monosomies by karyotyping several metaphases.

1. Study on the effects in maternal germ cells:

8-12 weeks-old females from mouse strain NMRI were pretreated with PMS (pregnant mares serum) and HCG (human chorionic gonadotrophin) 48 h later for synchronized ovulation. Females were exposed to 0,20 or 200 rad exactly 3 h after HCG injection during the radiation-sensitive preovulatory phase, and mated afterwards with untreated males of the same age and strain. Vaginal plugs - an indication of fertilization - were checked on the next morning and fertilized females were sacrificed 9.5 days p.c. (day of plug is day 0.5). Embryos were prepared cytologically and chromosomes analysed with the aid of G-banding. At least one karyotype was prepared from each embryo.

Altogether 90 embryos were karyotyped from matched control females: 46 embryos showing 40,XX and 43 embryos showing 40,XY. One embryo was triploid: 60,XXX. There was no deviation from the expected approx. 1:1 sex-ratio. Altogether 95 F_1-embryos were karyotyped successfully after maternal exposure to 20 rad: 45 embryos with 40,XX; 48 embryos with 40,XY and again one triploid embryo with 60, XYY, which in addition carried an unbalanced translocation between chromosome 1 and 3 (partial monosomic for No. 1 and partial trisomic for No. 3). Another embryo was tetraploid with 80, XXYY.

Maternal irradiation resulted in a high embryonic lethality. Only 22 implanted embryos and most of them severly retarded in development, were detected and prepared: 12 embryos with XX and 10 embryos with XY sex-chromosome constitution. So,

neither after 20 rad nor after 200 rad a significant deviation of the expected sex-ratio was detected.

Two embryos from the 200 rad-group were aneuploid: one monosomic for No. 19 and the other trisomic for No. 17. The first mentioned embryo was, in addition, affected by an unbalanced chromosome translocation between No. 1 and X. Altogether, only unbalanced translocations (5) were detected after maternal irradiation: 40,XX; -2,+t (1,2); 40,XX;-1,+t (1,2); 40,XX; -2,+t (2,3); 40,X;-X,+t (X,1) as well as the above mentioned embryo with 39,XY;-19,-1,+t (1,X). Incidence figures for translocations are 1.1 % after 20 rad and 22.7 % after 200 rad.

These studies on maternal exposure indicates that X-rays induce aneuploidy as well as structural chromosome anomalies during the final, sensitive, stage of oogenesis. The induced anomalies can be transmitted to the next generation and thus may increase chromosome anomalies in the F_1-offspring. Extrapolated to the human situation: maternal exposure would increase the risk for abortions - due to unbalanced karyotypes - and also for trisomies and monosomies which are compatible with postnatal life.

The unexpected finding was that maternal exposure produced only translocations in F_1-embryos in an unbalanced condition, which most likely can be explained by the characteristic pattern of oogenesis: the oocytes rest in a diploid condition until shortly before ovulation and one half of the chromosome set is separated into the 1. polar body.

2. Study on the effects in paternal germ cells:

The experimental condition for irradiation, handling the animals and preparing the embryos were the same as in the first study, only that males were irradiated with 0,20 or 200 rad and were mated consecutively to non-irradiated females for a period of 57 days.

Altogether, 1399 F_1-embryos were analysed and karyotyped during this large-scale experiment: 211 embryos from control females, 570 from the 20 rad-group and 618 from the 200 rad-group.

Table 1: Aneuploidy in 9.5 day-old mouse embryos after paternal irradiation with 0,20 or 200 rad

dose	F_1 embryos from days after irradiation (aneuploid/total)							
(rad)	2-8	9-15	16-22	23-29	30-36	37-57	Total	%aneuploid
0							0/211	0.0
20	0/123	0/61	2/52	1/76	1/64	2/194	6/570	1.1
200	3/156	0/69	2/19	2/67	1/72	0/245	8/618	1.3

The aneuploid karyotypes are:

- 20 rad: 41,XX; + 11 (2x); 39,XO; (2x); 41,XY;+ 2; 60,XXY
- 200 rad: 60,XXY (3x); 39,XO; (2x); 39,XY; -8; 41,XYY; 41,XY; + 13; 41,XY; + 14

The four aneuploid embryos from days 16-22 are all from males fertilizing females 22 days after irradiation. It is reasonable to assume that X-rays were effective during the last phases of spermatocytes, e.g. during the second meiotic division.

The incidence of aneuploidy in F_1-embryos from matings 16 to 36 days after irradiation is significantly higher compared to controls ($P < 0.05$). This is the first study which demonstrates cytogenetically that paternal exposure to X-rays increase the risk for aneuploidy in the following F_1-generation. The effect, however, is not very pronounced and we did not observed a clear-cut dose effect relationship. We did not observe an increase of triploidy in our material.

Interesting in this context - even when the finding is not statistically significant - is our observation of two aneuploid embryos from matings of days 37-57, i.e. after premeiotic

exposure to only 20 rad. No aneuploidy was detected in this group after the high dose of 200 rad. A similar observation was made during the study with pronuclei. This question of radiation induced aneuploidy in spermatogonia at low, but not high doses, seem to be important not only for the purpose of risk estimation in a population but is also important for genetic counselling of individuals exposed previously. Therefore we try to continue our study on stem-cell exposure.

The results of karyotyping and analysing for structural anomalies are given in table 2.

Table 2: Structural anomalies in 9.5 day-old mouse embryos after paternal exposure to X-rays.

dose	F_1-embryos from days after irradiation (unbalanced/balanced/total)						
(rad)	2-8	9-15	16-22	23-29	30-36	37-57	Total
0							0/211
20	0/123	0/61	0/52	0/76	0/64	0/194	0/570
200	0/5/156	2/7/69	2/0/19	1/0/67	1/0/72	1/235	19/618

No structural anomalies were detected in F_1-embryos after paternal exposure to 20 rad, but altogether 3.1 % F_1-embryos after exposing various stages of spermatogenesis to 200 rad. 1.1 % of these embryos were chromosomally unbalanced, whereas 1.9 % carried a translocation in a balanced condition. 18 of these anomalies were translocations and only 1 was considered to be a deletion on No. 9. Our incidence of balanced translocations in 9.5 day-old F_1-embryos is in good agreement with those data obtained with the test for hereditary translocations in the F_1-offspring obtained in different laboratories.

One embryo carried an unbalanced translocation after premeiotic (day 38) irradiation.

To have a better estimation of the primary effects in spermatogenesis we used the same experimental conditions again but analysed the F_1-generation immediately after fertilization in zygotes at the pronucleus stage.

We were not yet able to standardize the G-banding technique in this material: the efficiency of good preparations with high banding quality was not constantly high enough. Therefore chromosome analysis was done after standard staining.

Table 3: Chromosome anomalies in mouse pronuclei after paternal exposure to X-rays.

dose (rad)	Pronuclei from days after irradiation (abnormal/total)						
	2-8	9-15	16-22	23-29	30-36	37-277	Total
0							2/199
20	2/77	3/45	3/42	1/44	3/40	2/75	14/323
200	3/47	9/43	10/48	2/38	4/29	1/97	27/302

No structural anomaly was detected under these conditions in controls and only 2 of them (1.0 %) were hyperploid. Hypoploidy was not considered here, because only one metaphase is available and this type of aneuploidy can therefore be due to preparational artifacts. 1.6 % of the pronuclei were hyperploid in the 20-rad group and in the 200 rad-group this incidence was 4 %. The latter incidence is significantly higher ($P < 0.05$) compared to the controls. The study with pronuclei therefore corroborates the above mentioned study with 9.5 days-old F_1-embryos and underlines that X-rays may induce nondisjunction even during spermatogenesis.

As indicated in table 3, we observed a rather high number of structurally abnormal pronuclei after irradiation. Types of anomalies observed: small and large marker chromosomes, centric and acentric ring chromosomes, fragments, as well as micronuclei together with a hypoploid metaphase. The latter observation indicates chromosome loss during early cleavages.

The following conclusions can be drawn:

1. X-rays induce non-disjunction during mouse oogenesis and spermatogenesis, thus increase the incidence of aneuploidy (monosomy, trisomy) in the F_1-generation after exposure. Chromosome loss may produce monosomy in same embryos.

2. No clear-cut dose effect relationship for radiation-induced non-disjunction was detected so far.

3. Irradiation of preovulatory oocytes produce predominantly F_1-embryos with unbalanced structural anomalies,but no anomalies in a balanced condition. Exposure of spermatogenesis increase the risk for both, balanced as well as unbalanced translocations in F_1-embryos. Unbalanced predominate after exposure of spermatocytes, whereas in later stages balanced reciprocal translocation are preferably induced.

4. The incidence of anomalies is higher in pronuclei compared to 9.5 days-old F_1-embryos after the same kind of treatment. This indicates selection of chromosomally unbalanced embryos.

5. The finding of aneuploid F_1-embryos (pronuclei and 9.5 days-old embryos) after exposure of premeiotic germ cell stages to the rather low dose of 20 rad requires further consideration.

Publications:

1. I. Hansmann:
The induction of non-disjunction by irradiation in mammalian oogenesis and spermatogenesis.
Mutation Res. 1979, 61, 69-76

2. I. Hansmann:
Clustering of chromosomal aneuploidy and tracing of nondisjunction in man.
Environ, Health Perspect., 1979, 31, 23-25

3. I. Hansmann, H.-D.Probeck:
Detection of nondisjunction in mammals.
Environ, Health Perspect., 1979, 31, 161-165

4. I. Hansmann, R. Zmarsly, H.-D. Probeck, J. Schäfer, J. Jenderny:
Aneuploidy in mouse fetuses after paternal exposure to X-rays.
Nature 1979, 280, 228-229

5. H.-D. Probeck, J. Jenderny, I. Hansmann:
The induction of non-disjunction in mammalian oocytes by X-rays.
Mutation Res. (inpress)

6. H.-D. Probeck, J. Jenderny, I. Hansmann:
Genetic risk after exposure of maternal and paternal germ cells to X-rays.
Int.J.Nuclear Med.Biol. (inpress)

Vertragspartner der Kommission: Gesellschaft für Strahlen-und Umweltforschung mbH,8042 Neuherberg, Ingolstädter Landstr. 1

Nr. des Vertrages: 205-76-1 BIOD
Leiter der Forschungsgruppe: Prof.Dr.W.Pohlit, Abteilung Biophysikalische Strahlenforschung, 6000 Frankfurt/Main, Paul-Ehrlich-Str. 20, Telefon 0611/6303311

Allgemeines Thema des Vertrages: Direct and indirect radiation effects on DNA of eukaryotic cells and their repair

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Titel des Projets Nr. 1:

Direct and indirect radiation effevts on DNA of eukaryotic cells and their repair

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Dr.D.Frankenberg, Dr.M.Frankenberg-Schwager, Dipl.Phys. D.Blöcher, Dr.H.Schorn

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The main results obtained during the course of the EURATOM programme are summarized. The relevance of these data for radiation protection is given at the end of the report.

One to two unrepaired DNA double-strand breaks (dsb) per cell per lethal event were measured. We conclude that dsb are potentially lethal lesions.

This conclusion is based on the following experimental results obtained with diploid yeast cells. The RBE-and OER-values calculated from exponential dose response curves of the homozygous rad 52 mutant deficient in dsb repair were found to be similar to those for dsb measured immediately after irradiation with 30 MeV electrons or 3.5 MeV alpha particles under both oxic and anoxic conditions (Table I). Furthermore the number of initial dsb per cell per lethal event for these different irradiation conditions ranged between 1.2 and 1.8 (Table I). Since wild type (wt) yeast cells which exhibit a shouldered dose response curve are proficient in the repair of dsb, these lesions can be regarded in wt cells as potentially lethal lesions (PLL).

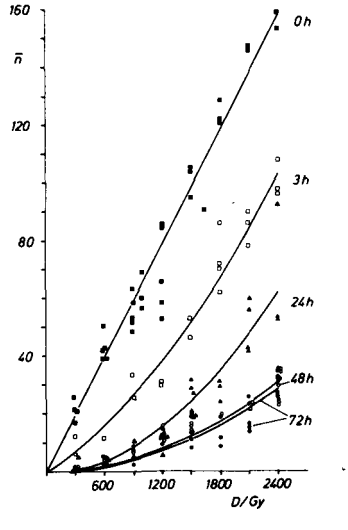
The shoulder of dose response curves with immediate plating is due to repair of PLL (dsb) within a restricted time period.

The shoulder of dose response curves obtained with diploid wt cells at the G1/S border is only 1/3 of the shoulder width of early Go phase cells. When irradiated G1/S border cells are prevented from proceeding through the cell cycle,their shoulder width approaches that of early Go phase cells. This result, together with those mentioned above, lead to the conclusion that the shoulder of dose response curves is due to repair of Poisson-distributed PLL (dsb) during a restricted time period between plating cells on nutrient agar and an unknown point in the cell cycle beyond which no further repair of PLL (dsb) is possible. Accumulation of sublethal damage, multi-hit, mulit-target theories, and pool theories are in contradiction to these experimental findings.

Table I: RBE- and OER-values for survival and induction of dsb. The number of dsb per cell per lethal event under different irradiation conditions.

		survival	dsb
RBE	O_2	2.o ± 0.1	2.6 ± 0.1
	N_2	5.4 ± 0.4	5.3 ± 0.4
OER	30 MeV electrons	2.4 ± 0.1	2.8 ± 0.1
	3.5 MeV alpha particles	0.9 ± 0.1	1.3 ± 0.1

Irradiation condition	dsb per cell per lethal event
30 MeV electrons, O_2	1.4 ± 0.1
30 MeV electrons, N_2	1.3 ± 0.1
3.5 MeV alpha particles, O_2	1.8 ± 0.2
3.5 MeV alpha particles, N_2	1.2 ± 0.2



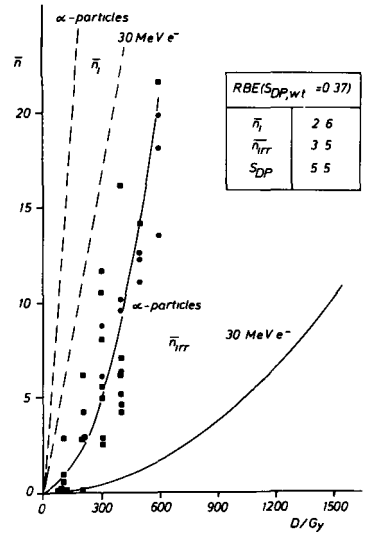


Fig.1: Mean number of dsb per cell as a function of dose for various periods of incubation of irradiated cells under nongrowth conditions at 30°C. The solid lines represent the best fit to the experimental points as calculated by regression analysis.

Fig.2: Mean number of dsb per cell irreparable under nongrowth conditions after irradiation with 3.5 MeV alpha particles and 30 MeV electrons ($\bar{n}_{irr}$). Furthermore, the initial dsb induced by these radiations are also presented ($\bar{n}_i$). The RBE-values at the level of 37% survival after delayed plating (DP) are shown in the inset for the initial and irreparable dsb and for survival after DP.

The relationship between the number of initial dsb and dose is linear. Dsb are induced exclusively by direct radiation action; indirect radiation action (action of the radicals of water radiolysis within cells) is negligible.

A linear relationship between the number of initial dsb and dose was found under oxic and anoxic conditions after irradiation with 30 MeV electrons and 3.5 MeV alpha particles. N_2O gassing changes the radical spectrum within cells such that the yield of OH radicals per dose is doubled. Since the number of initial dsb per dose after 30 MeV electrons was not changed in the presence of N_2O it was concluded that indirect radiation action does not contribute to the lethality of cells.

For different cell systems (bacteria, yeast, mammalian cells) it has been demonstrated in the last few years that single-strand breaks (ssb) in the nuclear DNA play a minor role for lethality. Thus the evaluation of direct and indirect radiation actions in the production of ssb was no longer relevant. These considerations led us to cancel the investigations of direct and indirect radiation actions in isolated yeast nuclei and chromosomes as well as the localization of radioprotective agents within cells. Instead of this further investigations on the induction of dsb and their repair were thought to be much more relevant for radiation protection since we now consider dsb to be the primary events in the DNA leading to chromosomal aberrations.

Dsb are repaired under nongrowth conditions. The linear relationship between initial dsb and dose (30 MeV electrons) is converted into a linear-quadratic relationship between irreparable dsb and dose during cell repair (Fig.1). Irradiation at extremely low dose rate reduces the quadratic term drastically. It was concluded that at high dose rate irreparable dsb are induced by interaction of reparable dsb (PLL). By analogy with the results obtained in survival studies it is proposed that the linear component of the relationship between irreparable dsb and dose is due to a single track of an electron of medium energy (2 keV up to 30 keV), whereas the quadratic component is caused by the cooperative effect of track ends (electrons with kinetic energies of about 1 keV) each of which is produced by a separate traversal of a slow electron in the yeast nucleus.

These results are in agreement with the suggestion that repair of dsb involves a recombinational process between two homologous chromosomes in the region of a dsb. It requires that one of these chromosomes is intact, i.e. has no dsb, in the corresponding DNA region. When two dsb are induced in the corresponding regions of both homologous chromosomes neither of these breaks is reparable.

The effect of changing radiation quality (LET) on the induction and repair of dsb was investigated using 30 MeV electrons and 3.5 MeV alpha particles. Dsb were induced linearly with dose both for sparsely and densely ionizing radiation. As was found for electron irradiated cells the linear relationship between initial dsb and alpha dose was converted into a quadratic function, with perhaps a small linear component, by repair of dsb.

The RBE-value of alpha particles for inducing irreparable dsb was found to be 3.5, higher than the RBE-value for the sum of reparable and irreparable dsb, which was 2.6. This means that densely ionizing radiation induces a greater number of both reparable and irreparable dsb per dose than does sparsely ionizing radiation, although the proportion of irreparable dsb was larger after alpha particle irradiation. Since the RBE-value increases during repair of dsb, it is inadmissible to relate the RBE-values of initial dsb to the RBE-values found for survival of cells proficient of dsb repair (Fig. 2).

Identical biphasic kinetics of dsb repair under nongrowth conditions were found after irradiation with 30 MeV electrons and 3.5 MeV alpha particles if corrections were made for irreparable breaks and for RBE. This suggests that the type(s) of reparable dsb are the same for both radiations. Thus the difference in effectiveness between sparsely and densely ionizing radiations may result only from the difference in the relative numbers of reparable and irreparable dsb per dose.

Yeast cells are the only eukaryotes in which induction and repair of dsb can be studied at the same radiation doses which are used in survival studies. In contrast to mammalian cells, for yeast it is possible to deduce that one or a few dsb per cell leads to cell death. Thus yeast cells provide a unique possibility to evaluate in a eukaryotic cell system, genetic and cellular effects at such low doses and at such low dose rates that only very few dsb per cell are produced. Data which have been obtained so far in yeast cells seem also to be relevant for mammalian cells.

Liste der im Rahmen des EURATOM-Vertrages Nr. 205-76-1 BIOD veröffentlichten Arbeiten:

1. Frankenberg,D.,Frankenberg-Schwager,M., Grundler,W., Krüger, E.H., Paretzke, H.G., Pohlit, W. and Rimpl, G., In: Sixth Symp.Microdosimetry (Eds. J.Booz, H.G.Ebert), Brüssel, Harwood Academic Publishers Ltd., 1978, 121
2. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Adamczyk, C., In: Proc.14th Ann.Meet.Europ.Soc.Radiat.Biol., Jülich, 1978, Abstract no. B2/26
3. Frankenberg-Schwager, M., Frankenberg,D., Blöcher, D. and Adamczyk, C., 1979, Int.J.Radiat.Biol.,36, 261
4. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Adamczyk, C., In: The Molecular Biology of Yeast, New York, Cold Spring Harbour Laboratory, 1979, 230
5. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Adamczyk, C., In: Ann. Meeting Deutsche Gesellschaft für Biophysik (Eds. G.Adam, G.Stark) Berlin, Springer Verlag, 1979, 118
6. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Adamzcyk, C., 1980, Int.J.Radiat.Biol., 37, 207
7. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Adamczyk, C., 1980, Rad.Res. 82, 498
8. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Adamczyk, C., 1980, Radiat.Environm.Biophys. 17 313
9. Frankenberg,D., Frankenberg-Schwager, M., Blöcher, D. and Adamczyk, C., In: 7th Sympos.Microdosimetry, 1981, in press
10. Frankenberg, D., Frankenberg-Schwager, M., 1981, Int.J. Radiat.Biol., in press
11. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Adamczyk, C.: Effect of dose rate on the induction of DNA double strand breaks in eukaryotic cells, sumitted to Rad.Res.
12. Frankenberg, D., Frankenberg-Schwager, M., Blöcher, D. and Harbich, R.: Evidence for DNA double-strand breaks as the critical lesions in yeast cells irradiated with sparsely or densely ionizing radiation under oxic or anoxic conditions. Submitted to Rad.Res.
13. Frankenberg, D., Frankenberg-Schwager, M., Blöcher, D. and Adamczyk, C.: OH radicals are not involved in the DNA double-strand breakage in irradiated yeast cells. In preparation
14. Frankenberg-Schwager, M., Frankenberg, D., Blöcher, D. and Harbich, R.: The RBE-values of initial and irreparable DNA double-strand breaks in yeast cells irradiated with 3.5 MeV alpha particles. In preparation.

Contractant de la Commission : Université Catholique de Louvain
N° du contrat : 157-76-1 Bio B
Chef du groupe de recherche : André GOFFEAU
Thème général du contrat : Radiobiology of mitochondrial DNA

Titre du projet n° 1 : Mitochondrial bioenergetics and oxidative metabolism of the irradiated and non irradiated *uvsρ* strains of *Saccharomyces cerevisiae*

Chef du projet et collaborateurs scientifiques : A. Goffeau, M. Briquet B. Crosby, A.-M. Colson et collaborations occasionnelles de E. Moustacchi et D. Tyteca.

The research project proposed in 1976 states that oxidative phosphorylation and non-phosphorylating enzyme activities in isolated mitochondria from UV sensitive, rho$^-$ producing strains (*uvsρ*) of mitochondrial and nuclear heredity will be pursued. It was proposed that the reactivity of the mitochondrial ATPase to the mutagenic agent ethidium bromide would be studied. Finally it was decided to investigate the effect of UV irradiation, photoreactivation and dark liquid holding on these processes. The project has been terminated in 1979 after the following results and conclusions were obtained

1. Oxidative metabolism of UV sensitive, mitochondrial mutations (rho$^-$) producing yeast strains.

We are able to provide a satisfactory explanation of the slow growth on non-fermentable substrates of the strain UVSρ72 of *Saccharomyces cerevisiae*. As described by Moustacchi, the mitochondrial DNA of this strain is hypersensitive to UV damage as expressed by induction of the mitochondrial rho$^-$ mutation. We have shown that previous claims of a modification of mitochondrial ATPase in this strain, by Bastos and Mahler, J. Biol. Chem 249, 6617-6627, 1974) cannot explain its slower growth on non fermentable substrates. We have devised a mathematical model which accounts for slower growth of UVSρ72 on the sole basis of

the continuous and elevated rho^- production in the mutant. This model has been verified experimentally and been generalized to any microbial population subject to constant and high rates of any type of stable mutation which results in slow growth.

2. Reactivity of the mitochondrial mutagenic agent ethidium bromide with mitochondrial ATPase

This study was induced by the model of Bastos and Mahler that mitochondrial ATPase was involved in the mutagenicity of ethidium bromide for mitochondrial DNA. Bastos has shown (J. Biol. Chem. 250, 7739-7746, 1975) that ethidium bromide binds to a membrane component of the mitochondrial ATPase. In contrast, we have observed that ethidium bromide inhibits the soluble part of mitochondrial ATPase in its purified form. Our data indicate a target for ethidium bromide located at a non catalytic site of the mitochondrial ATPase. The mutagenic effect of ethidium can be simply explained by a decrease of mitochondrial ATP due to inhibition of oxidative phosphorylation via the soluble part of mitochondrial ATPase. As mentioned above the modified reactivity of the mitochondrial ATPase of the strain UVS_p72 modified in repair of mitochondrial DNA, could not be reproduced. Therefore model mechanism which promote a direct role of mitochondrial ATPase in the repair or in the mutagenicity of mitochondrial DNA (Mahler and Bastos, Proc. Natl. Acad. Sci, 71, 2241-2245) has no longer any experimental support. In other words due to a series of irreproducible data of other authors, we have spent two years of work accumulating negative data. At least the situation was clarified and these explorations were stopped.

3. Effect of the bioenergetic metabolism on liquid holding repair

Although several distinct repair pathways for mitochondrial and nuclear UV damages have been identified in *Saccharomyces cerevisiae* in no case have the responsible gene products and their cofactors such as dependence upon ATP or specific ions, been identified. Survival enhancement during Liquid Holding Recovery (LHR) is known

to depend upon an active cellular respiration (Patrick *et al.*, 1964). Studies using inhibitors which were active in blocking LHR such as dinitrophenol, azide ion, cyanide ion and anaerobiosis reached the conclusion that ATP may be involved as a cofactor for survival repair. Kiefer (1976) observed that repair processes were coincident with a rise in glycolytic activity, and that respiration following UV treatment could not be positively correlated with a repair capacity. High glycolytic activity in glucose-repressed cells of diploid yeast has been shown to enhance survival following UV treatment. These results were interpreted as a preferential survival for those cells which derive ATP from glycolysis rather than from oxidative phosphorylation (Atsuta and Okajima, 1976). In addition to an apparent ATP requirement, post-irradiation cytoplasmic protein synthesis is required to enact survival and rho-minus LHR, although this is not the case for Negative Liquid Holding Recovery (NLHR, or an increase in the proportion of the mitochondrial DNA deletion mutant rho-minus during liquid holding) for rho-minus in stationary phase cells (Heude *et al.*, 1975). Mitochondrial protein synthesis was shown necessary for rho-minus and survival LHR only in logarithmic phase cells, whereas inhibition of mitochondrial protein synthesis during LHR had no effect on either survival LHR or rho-minus NLHR in stationary phase cells (Heude and Chanet, 1975).

We have sought to explore the following : 1. The effect of compartmentalisation of ATP between the mitochondrial and cytoplasm upon LHR from UV damages, using an inhibitor of mitochondrial adenine nucleotide translocation such as Bonkrekic acid (Subik *et al.*, 1972), in conjunction with inhibitors of mitochondrial electron transport and oxidative phosphorylation in wild type N123 and in the mutant UVSρ72 modified in the repair of mit DNA to UV damage (Moustacchi and Enteric, 1970). 2. The dependence of survival and rho-minus LHR upon mitochondrial and cytoplasmic protein synthesis in N123 and UVSρ72 as studied by the effects of chloramphenicol and cycloheximide.

Our result can be summarized as following. 1. The primary effect of

oligomycin was exerted upon the process(es) or rho$^-$-minus repair. 2. The primary effect of Antimycin A (in combination or not with Bonkrekic acid) was to alter the repair of survival (nuclear DNA) damages. 3. The effect of glucose is highly dependent upon the conditions in terms of presence of inhibitors, since a combination with Antimycin A and Bonkrekic acid served to restore survival LHR. Yet, glucose reduced survival LHR while at the same time stimulated rho-minus appearance (both whole and whole PLUS sectored) during liquid holding recovery of N123 cells. 4. Inhibition of cytoplasmic protein synthesis using cycloheximide in logarithmic cells of haploid UVSρ72 resulted in abolition of survival LHR as was the case for N123 haploid cells. However, whereas NLHR was reduced in stationary phase cells, in this case the rho-minus (whole and whole PLUS sectored) induction was increased suggesting that a cytoplasmically translated protein was required for repair of potential rho-minus cells. The response of UVSρ72 logarithmic cells to chloramphenicol resembled closely that of N123 logarithmic phase haploids in that survival LHR was abolished, but no effect was detected upon rho-minus induction during the liquid holding incubation.

These data are consistent with the following notions. 1. The ATP-dependence of LHR for survival and rho-minus is based upon an energetic requirement of controlled DNA degradation processes (postulated repair endonucleases) during the course of repair. NLHR for rho-minus in stationary phase cells involves a degradation of mitochondrial DNA in an ATP-dependent manner. The ATP required for both events is produced in sufficient quantities during liquid holding incubation when mitochondrial oxidative phosphorylation is functional. 2. Positive LHR for both survival and rho-minus requires the post-UV synthesis of some product of cytoplasmic translation. This peptide is crucial to successful DNA repair, and is either not constitutive in the cell, or is of short half-life. 3. The controlled positive LHR for survival and rho-minus in haploid cells involves a product of mitochondrial protein synthesis as a component of the repair process (either regulatory or structural). As for the hypothetical cytoplasmically-synthesised

component, the mitochondrial peptide is either not constitutive or has a rapid turnover in the cell. The inhibition of mitochondrial protein synthesis induces a further loss of control over rho-minus inducing damages in mitochondrial DNA of N123 stationary phase cells during LHR, as monitored by appearance of cells of UVSp72 suggests that this strain is a constitutively "decontrolled" mutant for induction of rho-minus NLHR and that the control factor is a mitochondrial peptide.

In conclusion :
The project one concerning the bioenergetics of dark repair of UV damage to yeast mitochondrial DNA led to clear conclusions and to a new model mechanism. This study was terminated in 1979 because we feel that the limit of the physiological approach has been reached. Only new investigations at the molecular level of enzymes involved in this dark repair of UV damage would now permit to progress in this domain and to control the validity of the model proposed to explain the physiological data. Such molecular attack would need a considerable new imput of funding which does not seem possible in the present state of priorities. Indeed, we understand that X and γ rays damages are more relevant to radioprotection of man than UV damages.

Titre du projet n° 2 : Genetical and biochemical analysis of repair of irradiated mitochondrial DNA in yeast.

Chef du projet et collaborateurs scientifiques : F. Foury, A. Goffeau B. Crosby, L. Bianchi

The research project proposed in 1976 states that the characterisation of mutants from *S. cerevisiae* with UV sensitive mitochondrial DNA will be pursued. It has also been decided that our effort will concentrate on the study of repair of yeast mitochondrial DNA to ionizing radiations. It was hoped that among the mutants, some will be deficient in the recombination of mitochondrial DNA. It was ascertained also that some mutations will be of mitochondrial inheritance.

In 1977, screening of spontaneous mutants resistant to ρ^- induction by ethidium bromide shows a clear relationship between ρ^- induction by UV and ethidium bromide resistance. In other words, this method has allowed the isolation of new mutants with an altered repair of mitochondrial DNA after UV irradiation. Clear quantification of mutation rate has been carried out thank to a mathematical model developed by Dr. Tyteca (Louvain-la-Neuve). This was an important development of our research project. In addition, it has been found that the mutant UVSρ72 isolated by E. Moustacchi exhibits a mutator phenotype for the auxotrophic marker his1. The pleiotropic phenotype of the mutant UVSρ72 suggests complex relationship between nuclear and mitochondrial repair pathways.

Since 1978, we have focused our research mainly on the repair of mitochondrial DNA subject to ionizing radiations. We have tried to isolate as many mutants as possible which present alterations of mitochondrial DNA repair. After four mutagenesis with ethylmethanesulfonate covering some 150,000 survivors, the following classes of mutants have been isolated.

1) Mutation strains of mitochondrial DNA

Among 450 gamma-ray sensitive mutants, five spontaneous mutators of mitochondrial DNA (gam) have been isolated. They determine five genes.

Two mutants were clearly sensitive to gamma-rays and were spontaneous mutators for only specific alleles of the mitochondrial DNA. The three other mutants were weakly sensitive (or insensitive) to gamma-rays and exhibited a mutator phenotype for all tested mitochondrial DNA alleles. The existence of the two types of mutants suggests that the spontaneous mutability of mitochondrial DNA in *S. cerevisiae* is dependent both on pathways specific for mitochondrial DNA and on pathways which control also the repair of nuclear DNA to gamma-rays.

2) Antimutator strains of mitochondrial DNA

Several mutants have been also isolated which exhibited both increased cell lethality and decreased induction of resistance to erythromycin, a mitochondrial DNA marker. Finally twelve spontaneous antimutators were selected. In 8 mutants, gamma-ray sensitivity and spontaneous antimutability were located at distinct independent genes. However in 4 mutants both traits segregated together, indicating that they were the product of a single mutation. All the mutants were spontaneous antimutators for both nuclear and mitochondrial genes. Depending on the allele and on the mutant, the spontaneous mutation rate could be decreased by a factor 2 or 100. In several mutants, the mutation rate was decreased only for forward mutations but not for the two reverse mutations examined ($met^- \rightarrow met^+$ and $his^- \rightarrow his^+$). The isolation of gamma-ray sensitive mutants with an antimutator phenotype for both nuclear and mitochondrial genes indicates that there are common controls in the repair of nuclear DNA and the spontaneous mutability of the nuclear and mitochondrial genomes.

3) $\gamma s\rho$ mutants

In 4 mutants, temperature shift from 30°C to 36°C induced ρ^- after several generations at the non permissive temperature. The induction of ρ^- was considerably increased by gamma-irradiation followed by incubation at 36°C for 24 hr. The synthesis of mitochondrial DNA was not affected at 36°C, nor was the synthesis of mitochondrial RNA. The degree of the the genetic information retained by the ρ^- varied from one mutant to the other but it was interesting to note that as in the

case of spontaneous ρ^- or UV and ethidium bromide induced ρ^-, the mitochondrial DNA located in oxi1-oxi2 and cob regions were preferentially retained, while the genetic information of the oxi3 and oli2 loci was preferentially lost. In the mutant GA19-10, ρ^- were not induced in the absence of irradiation. However gamma-rays followed by incubation at 30°C induced both cell lethality and ρ^- induction. Tetrad analysis showed that these two traits were produced by a single gene nuclear mutation. The induced ρ^- had retained a low degree of genetic information.
These 5 mutants determined five distinct nuclear genes.

In 1980, we have undertaken the enzymology of these mutants. We have found that in isolated mitochondria of one mutant, the degradation of newly synthesized DNA was similar to that of the parent in incubations at 25°C but was significantly decreased in a temperature shift at 30°C. An enzyme activity -possibly a mtDNase- involved in the degradation of mtDNA is therefore thermolabile in this mutant.

Conclusions

As proposed initially in the research project, new mutants modified in the repair of mitochondrial DNA have been isolated. All of them are nuclear. All together some 14 genes have been found to control the repair of mitochondrial DNA. Seven genes control both gamma-ray sensitivity and the spontaneous or induced mutability of mtDNA. Two mutants are spontaneous mutators for certain alleles of the mtDNA. Four mutants are spontaneous antimutators of both mitocondrial and nuclear genes. One mutant exhibits both increased cell killing and ρ^- induction after gamma-rays. Seven genes control specifically the spontaneous mutability or repair to gamma-rays of the mitochondrial DNA.

Our results emphasize the importance of the study of repair of mitochondrial DNA to gamma-rays. 1) Because at least seven genes control specifically the repair of mitochondrial DNA which in mammalian cells is a very important target, since severe lesions of this

DNA are not viable. 2) Because at least seven genes control both repair of mitochondrial DNA and of nuclear DNA, suggesting complex interrelationship between the two systems which are of importance to be considered in the study of general damage of a living cell by gamma-rays.

List of publications concerning yeast mitochondrial DNA and mitochondrial bioenergetics of the Laboratoire d'Enzymologie from 1976 to 1980

Many papers were only indirectly linked to the repair of mitochondrial DNA. However without the know-hows that has been developed through these papers it would not have been possible to make the contributions specifically related to mitochondrial DNA repair.

1976

M. BRIQUET
The transport of pyruvate in yeast mitochondria.
Arch. Int. Physiol. Biochim. 84, 584-585.

J. DELHEZ, J.P. DUFOUR and A. GOFFEAU
Comparaison des propriétés des ATPases mitochondriales et plasmiques chez la levure *Schizosaccharomyces pombe*.
Arch. Intern. Physiol. Biochim. 84, 602-603

F. FOURY, M. BOUTRY et A. GOFFEAU
Fonctions de transport de l'ATPase plasmique de *Schizosaccharomyces pombe*.
Arch. Int. Physiol. Biochim. 84, 618-619.

F. FOURY, J. DELHEZ, M. BOUTRY, J.P. DUFOUR and A. GOFFEAU
Role of the plasma membrane ATPase in the cellular uptakes of cations and amino acids in the yeast *Schizosaccharomyces pombe*.
Tenth Intern. Congress of Biochemistry, Hamburg 1976, Abstract 06-6-169.

A. GOFFEAU and Y. LANDRY
Compared properties of the oligomycin-sensitive ATPase in fungi with short and long mitochondrial DNA.
In "Genetics, Biogenesis and Bioenergetics of Mitochondria", W. Bandlow *et al.* (eds.), Walter de Gruyter, Berlin, New-York, pp. 303-308.

F. FOURY and A. TZAGOLOFF
Localization of mitochondrial DNA of mutations leading to a loss of rutamycin-sensitive adenosine triphosphatase.
Eur. J. Biochem. 68, 113-119.

A.M. COLSON, F. LABAILLE and A. GOFFEAU
A cytoplasmic gene for partial suppression of a nuclear pleiotropic respiration deficient mutant in the "petite-negative" yeast *Schizosaccharomyces pombe*.
Molec. gen. Genet. 149, 101-109.

M. BRIQUET, N. SABADIE-PIALOUX and A. GOFFEAU
Ziram, a sulfhydryl reagent and specific inhibitor of yeast mitochondrial dehydrogenases.
Arch. Biochem. Biophys. 174, 684-694.

A. GOFFEAU, F. LABAILLE, O. MOHAR and A.M. COLSON
Screening tests for suppressors of respiratory deficient mutants in *Schizosaccharomyces pombe* and model for a mitochondrial partial suppression of a nuclear pleiotropic strain.
In "Genetics and Biogenesis of Chloroplasts and Mitochondria (Bücher, Th., Neupert, W., Sebald, W. and Werner, S., eds) pp. 851-856, North Holland Publ. Co., Amsterdam.

B. CONVENT and M. BRIQUET
Modification by antimycin of the interaction of substituted urea with the cytochrome b-c_1 segment of the electron transport chain in *Saccharomyces cerevisiae*.
Tenth Intern. Congress of Biochemistry, Hamburg 1976, Abstract 06-2-176.

A.M. COLSON, F. LABAILLE, O. MOHAR and A. GOFFEAU
Mitochondrial suppressor of a nuclear pleiotropic respiratory-deficient mutant in the "petite-negative" yeast *Schizosaccharomyces pombe*.
Arch. Int. Physiol. Biochim. 84, 1057-1058.

1977

M. BRIQUET
Transport of pyruvate and lactate in yeast mitochondria.
Biochim. Biophys. Acta 459, 290-299.

M. BOUTRY, F. FOURY and A. GOFFEAU
Energy-dependent uptake of calcium by the yeast *Schizosaccharomyces pombe*.
Biochim. Biophys. Acta 464, 602-612.

A.M. COLSON, L. THE VAN, B. CONVENT, M. BRIQUET and A. GOFFEAU
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Vertragspartner der Kommission: Justus-Liebig-Universität Giessen

Nr. des Vertrags: 261-78-7 BIO D
Leiter der Forschungsgruppe(n): Prof. Dr. J. Kiefer

Allgemeines Thema des Vertrags:

Survival and mutation induction in Saccharomyces cerevisiae

Titel des Projekts Nr.

Effects of heavy ions on survival and mutation in Saccharomyces cerevisiae

Leiter des Projekts und wissenschaftliche Mitarbeiter:

J. Kiefer, J. Luggen-Hölscher, G. Mock, S. Rase, E. Schneider, F. Schöpfer, F. Zölzer

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This project was carried out to study the mutagenic action of very densely ionizing radiations. High energetic heavy particles are an important component of extraterrestrial radiation; they are also produced by accelerators for physical and biomedical experiments. The knowledge of their genetic action is therefore of fundamental interest and has great practical importance.

The yeast Saccharomyces cerevisiae was used as an eucaryotic model system which can be tested for a well defined mutation, namely the induced resistance to the arginine analogue canavanine. To establish the base line for this work mutation induction was studied with 80 kV X-rays. High LET experiments included ^{241}Am-α-particles and the accelerated ions Ar, Kr, Xe, Pb and U provided by the UNILAC at the Gesellschaft für Schwerionenforschung, Darmstadt, Germany. A haploid wild type strains 211-1a, was used. In the X-ray experiments aqueous suspensions were exposed in open petri dishes with vigorous stirring in gas tight enclosure. At the UNILAC the cells were exposed on membrane filters to a broad homogeneous beam of heavy ions. After exposure they were resuspended in water. Survival and

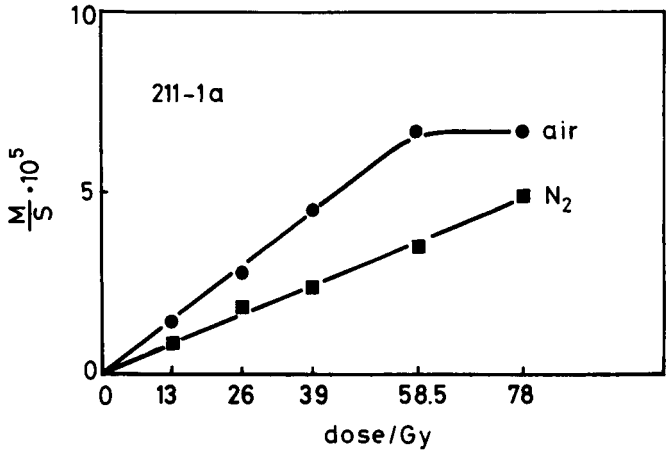

Figure 1: Mutation rate as a function of X-ray dose after exposure in air and nitrogen for strain 211-1a

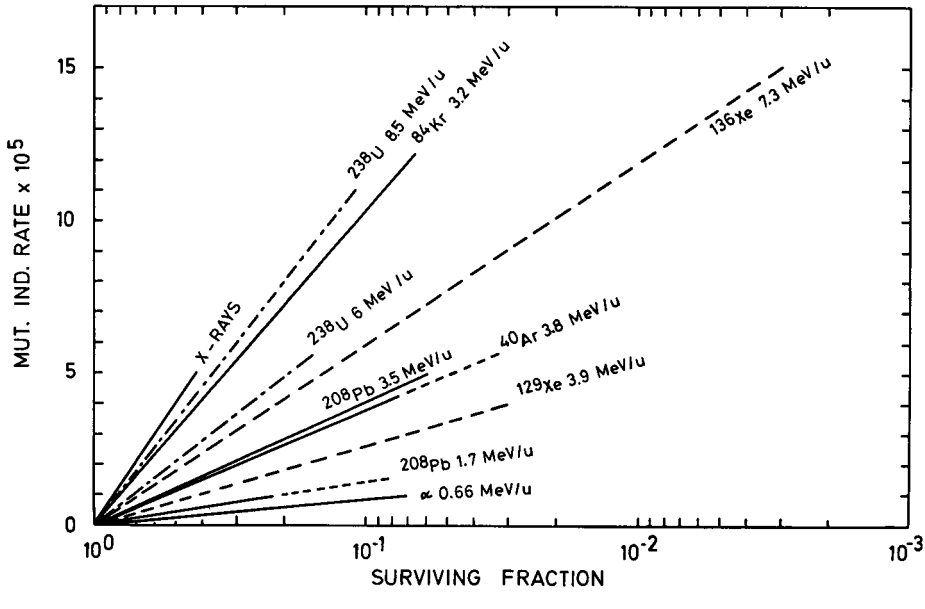

Figure 2: Mutation rates as a function of log survival for X-rays and ions of different energies (data points omitted for clarity)

mutation induction were than tested for colony forming ability. Special nutrient agar plates containing either the amino acid arginine or its analog canavanine were used as test media.

The mutation rate M/S was calculated as mutants per survivor after subtraction of the spontaneous mutation rate which was typically about $4 \cdot 10^{-6}$. Within the low dose region investigated there was always a linear relationship between induced mutation rate and dose. Figure 1 shows the results with X-rays. A clear oxygen effect is seen with an oxygen enhancement ratio of 2, which is the same as for survival. The oxygen enhancement ratio depends on O_2-concentration according to the Alper-Howard-Flanders formula. The parameters are not significantly different for survival and mutation induction: $m = 1.96 \pm 0.27$; $k = (27.8 \pm 11.1)$ µM O_2.

Figure 2 shows a plot of induced mutation rate versus the logarithm of survival for X-rays and different ions. It can be seen that in this plot the dependence is linear in all cases, the slopes however are different for the various radiation qualities. X-rays appear to be the most effective more so than the accelerated ions. Effectiveness increases with specific ion energy and comparing ions of equal energy it also increases with mass. This demonstrates the importance of wide-ranging δ-electrons. The number of these secondary electrons is much larger with ions of high velocity.
Effective cross sections σ_i for inactivation and σ_m for mutation induction are presented in the following table.

Radiation	specific Energy MeV/u	LET_∞ /keV·µm^{-1}	σ_i/cm^{-2} x 10^8	σ_m/cm^{-2} x 10^{12}
X-rays: 80 kV		3	-	-
^{238}U	8.5	13 160	5.5	2.7
^{84}K	3.2	4 880	1.45	6.7
^{238}U	6.0	14 100	4.55	1.4
^{136}Xe	7.3	6 600	3.7	0.94
^{208}Pb	3.5	12 790	3.5	0.64
^{40}Ar	3.8	1 930	1.25	0.21
^{129}Xe	3.9	7 550	3	0.34
^{208}Pb	1.7	12 460	1.54	0.1
α	0.66	160	0.5	0.125

The aim of this project was to obtain information about the mutation induction by very densely ionizing particle radiations. This aim was reached with respect to the induction of canavanine resistance (forward mutation CAN → can) in the haploid strain 211-1a. A second mutation (his → HIS) in another haploid strain is still to be investigated. A few mutation experiments under unaerobic conditions were performed. The results however have not yet been statistically validated.

The knowledge of the mutation induction by energetic heavy ions is of fundamental importance to radiation protection. Our results can help to evaluate the hazards of these particle radiations for which very little information is available.

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Rad. Environm. Biophys 18 (Suppl.), 14, 1980

Biology Group of DG XII at Ispra

Head of research team : M. DEVREUX

General subject of the research : Genetical and biochemical analysis of the radiosensitivity of eukaryotic cells.

The aim of the research was to contribute to the knowledge of the processes involved in the induction and repair of radiation damages, and particularly by low doses in complex eukaryotic cells.

The problem was tackled at both the genetical and the biochemical levels.

A. The genetic analysis of radiation effects on eukaryotic cells cultured in vitro.

Scientific staff: R. Cavalloro, M. Devreux, E. Magnien

A.1. Protoplast isolation and culture

The leaf protoplast system provides, from an homogeneous starting material, a culture of single cell clones with an extremely high plating efficiency.

Difficulties encountered in generalizing this valuable technique were progressively overcome by systematically modifying the experimental procedures in consistence with the local conditions. Finally, it is now possible to obtain repeatedly such high plating efficiencies to allow reliable radiobiological applications.

A.2. Caryological stability

In long term cultures, cell lines often demonstrate a poor caryological stability. In order to control this stability, cell lines coming from microspore-derived embryoids of Datura innoxia were maintained in vitro under 22 different hormonal combinations and periodically submitted to cytophotometric measurements. Among all the combinations tested, some of them, with a high cytokinin:auxin ratio, showed a tendency to select for the proliferation of preferentially low DNA-content nuclei. These results could be the consequence of two concomitant phenomena: the selective advantage of haploid nuclei due to their shorter mitotic cycle, together with an increased stimulation to mitotic division provoked by a higher cytokinin supplement.

A.3. Modification of cell radiosensitivity

The isolation of leaf protoplasts provides a system in which a homogeneous population of cells undergoes specific stimuli and comes across characteristic phases of dedifferentiation in a limited time.

The whole process was calibrated with respect to the nuclear phases using Nicotiana plumbaginifolia and it was found to be remarkably synchronous. Taking advantage of this favourable situation, certain defined stages before and after protoplast release were checked for their sensitivity towards γ-rays. Irradiated protoplasts from untreated leaves were definitely more resistant than leaves irradiated at identical dose levels. The radiosensitivity increased again during the first day of culture up to a maximum immediately before the resumption of DNA replication. The sensitivity of the late S nuclear phase returned to the initial level of the early culture. No simple explanation, but only speculations, could be advanced to justify the higher resistance of the protoplast compared to the initial leaf cell.

A.4. Genotypic variations of radiosensitivity

Among five fully homozygous genotypes of the same species, but of different origins, only one was identified as significantly more radiosensitive. Its different behaviour was confirmed by repeated dose-response and split-dose experiments. Its D_o is lower and its rate of recovery, when dose fractions are separated by resting intervals, is reduced.

Taking into account the absence of variability among the other four genotypes, our finding looks much more like a particular case of repair deficiency. Trying to verify this hypothesis, some tests monitoring the repair kinetics are presently performed, such as unscheduled DNA synthesis measurements or the rejoining of DNA strand-breaks.

A.5. Dose-effect relationships, RBE and split-dose effects

Protoplasts from two diploid Nicotiana species were used to investigate the effects of γ-rays and fast neutrons. The damage was assessed by scoring the relative plating efficiencies. The effects of a single acute dose as well as fractionated doses were analysed for both qualities of radiation. The dose-RBE relationships, expressing the effectiveness of fast neutron relative to γ-rays as a function of dose, appears similar to that of most animal systems, with higher values in the region of lower doses. Whereas fractionation of γ-ray doses allowed a substantial recovery for sufficiently long time intervals, the fractionation of 30 rad of fast neutrons in six doses of 5 rad separated by 1 hour intervals produces an increase in effect equivalent to that of an acute dose of 1208 rad of γ-rays. This could not be explained by cell progression and necessitated an additional hypothesis, possibly involving other mechanisms in the specific action of low doses of radiation.

A.6. First cell cycle after irradiation

The homogeneity of the leaf protoplast system can be improved by the separation of uniform sub-populations characterized by cytological and histological criteria, through tissue dissection followed by an iso-osmotic gradient cell purification. This procedure gives rise to a fairly good synchronisation in the initial cell progression in culture.

Using this system, it was possible to observe the variation of several parameters when 500 rad γ ray-irradiated protoplasts go through the successive phases of their first cell cycle.

Cytophotometric sections permitted the monitoring of the extension of a G_2 block. A correlation between nuclear volume and DNA content per nucleus in S phase was established in each case of irradiated and non-irradiated protoplasts. Tritiated thymidine pulse labelling helped to trace the kinetics of DNA replication, showing a transient lag-time restricted to the initial steps of incorporation in the irradiated cells.

In the same way, ^{3}H-uridine and ^{14}C-leucine incorporation put emphasis on spasmodic disorders associated with growth stimulation in irradiated cells.

In collaboration with the Genetic Institute of Pavia University, the polymerase and the uracil-DNA-glycosylase activities were measured various times after gamma-ray treatment and were shown to be active in plant cells as they are in any mammalian cell.

A similar approach has been started with asynchronous continuously growing carrot cell culture, which should permit the measurement of the accumulated effects of low chronic doses of ^{3}H β-irradiation. The uracil-DNA-glycosylase activity was also detected in this material.

A.7. Mutagenic effect of γ-rays

Taking advantage of the haploidy of androgenetic plants and cell lines, theoretically it is possible to score an unlimited number of end

point mutations, provided appropriate screening systems can be devised in each case. Therefore, a spectrum of very different genetic markers was retained.

For recognition of chlorophyll-deficient clones, a procedure based on a diminution of the sucrose content and an increase in illumination was defined, which helps to magnify the pigmentation differences between wild type and variants.

In order to prepare the selection for specific drug resistances, inhibition curves were drawn in the cases of 5-methyltryptophan, L-valine, 6-thioguanine, para-fluorophenylalanine and $KClO_3$.

Mutation experiments are in progress. Characterization of mutants will be pursued until complete regeneration of normal organisms.

A.8. DNA repair activities

Unscheduled DNA synthesis can easily be measured after UV-ray or gamma-ray irradiations of a protoplast population. Radiation-stimulated ^3H-thymidine incorporation, following 3 hours of incubation, was counted by liquid scintillation. No replication synthesis can take place with the conditions employed: in any case, almost 100% of the protoplasts are in the resting phase at the time of isolation. However, to assure complete inhibition of replication, the specific α-polymerase inhibitor aphidicolin was always added to the cultures (obtained through courtesy of F. Sala et al. who first identified its effect on plant DNA replication). The incorporated radioactivity of 1500 J/m^2 UV-ray irradiated protoplasts was 35 to 40 times higher than that of the unirradiated control. After 50 Krad gamma-ray irradiation, the increase of incorporated radioactivity was by a factor of 2 or 3 compared to the control, the lower counts in this case being probably due to the shorter patch of repair.

A procedure which should allow the direct lysis of protoplasts on the top of an alcaline sucrose gradient, and the further resolution of their DNA in molecular weight classes, is presently being improved for the study of the rejoining kinetics of *in vivo* strand breaks.

A.9. Insect cell lines for the study of radiosensitivity

Differential sensitivity of biological systems to various radiation qualities has been observed in many experiments. In general, fast neutrons and gamma rays have proved to produce largely different biological responses, and the relative response depends on the dose level of the radiation applied.

In this context, Med-fly (*Ceratitis capitata* Wied.) cells were tested by observing cell population growth and mortality. This test is of particular interest because the Med-fly cell lines have been established these last years in our laboratory from embryonic eggs 24 hours old.

Fast neutrons of 1.5 and 16 MeV were produced by a Van de Graaff KS 3000 accelerator, whereas ^{60}Co gamma rays irradiation was performed using a 220 Gammacell apparatus. Neutron doses ranged between 1.5 and 25 rad and for the gamma rays, dose values between 300 and 5000 rad were used.

The experimental results show a much higher radiosensitivity of these cells to fast neutrons relative to gamma ray radiation, and a clear dependence of the RBE values on neutron-dose levels. Furthermore, a certain influence of radiation quality was observed for the cellular damage, the DL_{50} values for the 1.5 and 16 MeV fast neutrons were 12.5 and 15.5 rad respectively.

Dose-effect relationships are been analyzed for both neutrons and gamma rays while first attempts to observe split dose effects of ionizing radiations are in progress with this material.

The DNA content of the cell nuclei was analyzed at different time intervals with a Barr and Stroud integrating microdensitometer.

The results showed that the unirradiated cell nuclei were distributed in two peaks. These peaks corresponded to the diploid G_1 and G_2 stages, while a limited number of nuclei (1.7%) showed a higher DNA content. After the irradiation the frequency of nuclei with a DNA content above the value of 4 C increased with the radiation doses.

Such Feulgen positive nuclear masses probably correspond to degenerating cells resulting from nuclear fusion or imperfect synthesis. After 5,000 rad the DNA content was widely dispersed indicating complete degeneration.

A.10. DNA polymerases in Insect cells

In collaboration with Drs. U. Bertazzoni and A.I. Scovassi of the CNR laboratory of Pavia, a biochemical analysis of insect cells proved to be extremely rich in DNA polymerizing enzymes, they provide a very interesting system to study DNA replication and repair in eukaryotic cells.

The presence of the three DNA polymerases: α, β and γ, both in Ceratitis capitata Wied. cultured cells and insect embryos, was confirmed.

The levels of these enzymes, followed at different times during embryogenesis, showed that α-polymerase increases at an early stage of egg development (in connection with the maximal rate of DNA synthesis) while β and γ polymerases reach their highest activities at later times. In particular, the level of γ-polymerase, corresponding to the mitochondrial DNA polymerase, after 24 hours increases at least 5 times with respect to the early embryonic phase (see Fig. 1).

Attention was focused on the characterization of the DNA polymerase β whose presence in the insect embryo has been reported by us for the first time.

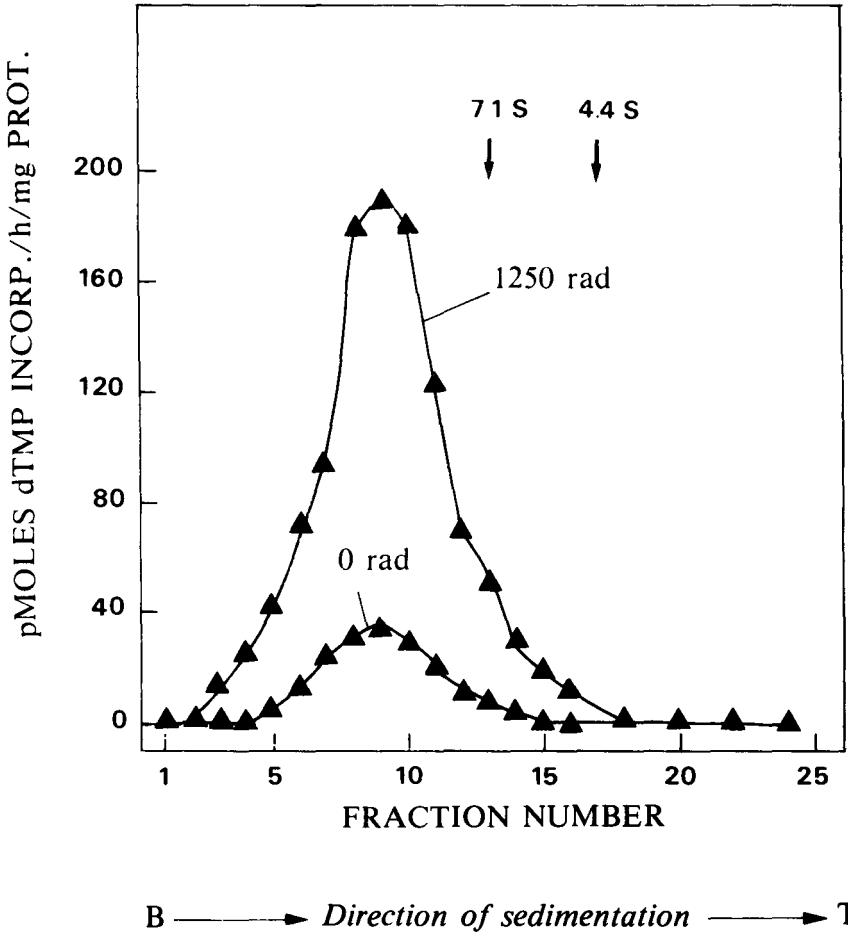

Fig. 1.- DNA POLYMERASE γ IN MED-FLY CELLS BEFORE AND AFTER γ-IRRADIATION

The β -polymerase is readily measurable in the crude extract which represents 20% of the total DNA polymerase activity, but is lost upon fractionation on sedimentation gradients.

The recovery of the β-activity by mixing experiments was obtained and the sedimentation value was about 7 S.

It seems, therefore, that the insect β-polymerase differs from the vertebrate enzyme (3.5S) by its higher molecular weight and its need for an activating factor.

The effect of different doses of ionizing radiations on the three DNA polymerases were also studied. The levels of α and β-polymerases do not show significant variations, while the level of γ-polymerase increases several fold with the irradiation dose. This notable increase in the mitochondrial DNA polymerase could be justified by the energy production needed for the DNA repair processes triggered by the irradiation.

This particular aspect, together with the high levels and coupling to an activator of the β-polymerase, which is involved in the DNA repair process, need to be further investigated in view of the striking insect resistance to ionizing radiations.

B. Biochemistry of DNA damage and repair

Scientific staff: F. Campagnari, L. Clerici, Myriam Talpaert-Borlé

B.1. DNA polymerases

The previously described DNA polymerase of the γ type, that had been isolated from the nuclei of bovine thymocytes, was further characterized. This enzyme and the isologous DNA polymeraseβ were assayed for their activities with X-irradiated DNA templates. Doses up to 1000 rads

had scarce effect on the priming properties of DNA. However, templates exposed to heavier doses (2000 rads and up) were not proficiently copied and the complementary replication of their nucleotide sequences did not go to completion. The results matched those previously obtained with the cytoplasmic DNA polymerase α also from calf thymocytes which still displays distinctive biochemical feature as compared to the similar deoxynucleotidyl transferase from cell nuclei. These mammalian enzymes for DNA synthesis recognize mildly irradiated DNA as functional templates, provided that 3'-OH initiating sites preexist or are made available by repair nucleases.

B.2. DNA ligase

The calf thymus enzyme firstly isolated in the Ispra laboratory was further purified up to 3000-fold over the crude tissue homogenate and was found devoid of contaminating nucleases. The ligase was assayed for its ability to work on X-irradiated DNA containing single stranded breaks and on synthetic polydeoxynucleotide substrates with an irradiated nucleotide chain at either the 3' or the 5' side of their sealable nick.

The enzyme specifically closed monohelical scissions carrying 3'-OH and 5'-PO_4 termini and was rather undiscriminative with regard to the structural integrity of the nucleotide sequences near the scissions. In the broken strands of irradiated DNA, the ligase rejoined adjacent polynucleotide chains still endowed with intramolecular damage.

When acting on DNA with altered base residues, such as the DNA exposed to X-rays and prior to the reactions promoted by other repair enzymes, the ligase restores the physical continuity of the double helical macromolecule but it contributes to fix premutational lesions in the genetic material.

B.3. "Proofreading" exonuclease

A 3'→ 5' exonuclease activity specific for single stranded DNA was found in DNA polymerase fractions prepared from calf thymocyte nuclei. This nuclease carried out the excision of mismatched nucleotides from the 3'-OH priming sites of synthetic template-initiator DNA structures in a cooperative manner with the complementary duplication of the template moiety by the DNA polymerase. The enzyme behaved as a "proofreading" exonuclease which was able to perform an editing function during DNA synthesis in vitro. The error-correcting nuclease remained apparently associated with the nuclear DNA polymerase of type α throughout the first steps of the isolation procedure. A number of biochemical properties, such as requirements for functional catalysis and specific responses to inhibitors, were also common to both enzymes. In the advanced stages of purification, the correcting nuclease either was lost or separated from the DNA polymerase which started breaking down into subunits and/or smaller size proteins.

The findings supported the assumption that mammalian cells possess a proofreading enzymatic system for faithful replication of DNA.

B.4. Uracil-DNA glycosylase

The enzyme removes uracil occasionally formed or incorporated in DNA. This glycosylase was firstly extracted from the nuclei of calf thymocytes and purified about 2000-fold over the crude thymus homogenate up to a stage where contaminating AP endonucleases and DNases were undetectable. The enzyme consisted of a small protein which combined specifically to polymeric nucleic acids with a preference for the double stranded forms and was able to split only the N-glycosyl bonds of deoxyuridyl units at the internal positions of polynucleotide chains. The glycosylase did not use free pyrimidines nor pyrimidine donors for base exchanges with uracil-DNA and for direct base insertions in apyrimidic DNA.

Although the reaction equilibrium was completely shifted to the hydrolytic side, the enzyme function was impaired when the concentrations of AP sites formed in DNA approached the values of Km's for the uracil residues of DNA substrates. This is a sort of modulation of the glycosylase catalysis by the ratio of its substrate and product structures in DNA. It might serve to control the accumulation of AP sites in cellular DNA and to couple the removal of uracil from DNA to the next step of the repair pathway, i.e. the AP endonuclease reaction.

Two uracil-DNA glycosylases were prepared from human placenta and comparatively studied. They were isolated from nuclei and mitochondria after cell disruption and purified 215-fold and 3000-fold, respectively, over the tissue homogenate. Both enzymes catalyzed essentially irreversible hydrolytic reactions and resembled in many properties the calf thymus glycosylase. The placental enzymes were not identical and differed in the following features: molecular size, tendency to form aggregates in diluted buffer solutions and sensitivity to inhibition by salts.

It appears that nuclei and mitochondria of human cells possess distinct systems for excision of uracil from their DNA.

B.5. Terminal deoxynucleotidyl transferase, TdT

The enzyme was routinely purified on a large scale and used for the biochemical preparation of DNA-like polynucleotides. TdT is a biochemical marker for immature precursors of T-lymphocytes in bone marrow and in the thymus cortex. An optimized assay method was standardized and adopted for determining the TdT content of human malignant lymphomas. The enzyme activity was abnormally elevated in the lymphoid tumors classified as lymphoblastic lymphomas. In these neoplastic tissues, forms of TdT with either high or low molecular weights were noted. The findings paralleled the reports about high levels of TdT in undifferentiated lymphoid cells from the blood of patients with certain forms of leukemia.

Antibodies against TdT were obtained in antisera of immunized rabbits and were purified up to 300-fold. The isolated antibodies were used to standardize immunofluorescence tests for detection of TdT-positive cells by microscopic analysis.

The TdT activity is a useful marker for transformed neoplastic clones of primitive lymphoblasts either disseminated in the blood or nested in solid tumors and with more sensitivity to cortisone treatment than to radiotherapy.

B.6. Biophysical studies

These investigations were joined efforts with scientists from the Joint Research Centre at Ispra.

Conformational changes induced by pH variations in a series of dT oligonucleotides dissolved in aqueous media were monitored by proton magnetic resonance, PMR, at high resolution. The data were correlated with the abilities of these compounds to act as primers for the polymerizing reaction catalyzed by terminal deoxynucleotidyl transferase.

The PRM method of measuring the relaxation enhancement of water protons in DNA solutions containing Mn^{2+} as a paramagnetic probe was used to study the exchanges between free water and the water linked to Mn^{2+} and interacting with DNA. The two types of water molecules exchanged within microseconds. It was also found that the hydrated Mn^{2+} ions have two binding sites in DNA. Mn^{2+} neutralized aspecifically the negative charges of two adjacent phosphate groups of DNA and combined with high affinity to the deoxyguanyl residues by forming chelation complexes between the N7 atoms and the phosphates. The strong interaction of Mn with the dG nucleotides in DNA distorts locally the regular bihelical conformation and might account for the mutagenic action of Mn.

A spectropolarimeter was developed for detecting the optical anisotropy induced in aqueous solutions of nucleic acids by a modulated electric field. The applied electric pulses aligned transiently the long

polynucleotide molecules which were randomly dispersed in the aqueous medium. The recorded signal of electrical birefringence differentiated clearly between double and single stranded forms of DNA in solution.

B.7. Tritium toxicity studies

The work was carried out in collaboration with scientists from the Joint Research Centre at Ispra and from the Department of Biochemistry of the University of Dublin.

Several types of polydeoxynucleotides and DNA molecules with tritium, ^{3}H, incorporated in the purine or pyrimidine residues were prepared. These polymers were kept in aqueous solutions for various periods and were monitored for both the exchanges of incorporated ^{3}H with the protons of solvent water and the radiation-induced decay of their macromolecular structure. Proper comparisons were established with non-tritiated and/or ^{14}C-labeled DNA molecules.

At equivalent radiation doses delivered to the overall DNA solutions, ^{3}H was more detrimental for the physicochemical integrity of nucleic acids than ^{14}C. However, the effect was partially artifactual since the definition of the real radiation doses to the critical targets, i.e. the hydrated DNA molecules, was difficult to estimate.

The ^{3}H atoms linked to the C8 positions of the purine bases of DNA echanged with the protons dissociated from solvent water at appreciable rates also under physiological conditions. The half-lives at 37°C for ^{3}H combined to the C8 of purines in poly(dA), poly(dG) and DNA ranged from one to only a few weeks and were comparable to those measured for the dissociation of the H-C8 bonds of dAMP and dGMP mononucleotides in PMR experiments. These data will contribute to correct the current notion that the hydrogen atoms linked to the C8 of purines in nucleic acids are "non-exchangeable" as assumed for the estimates of DNA contamination by HTO and tritiated compounds.

The toxicity of ^{3}H for mammalian cells was studied using the very sensitive system of mouse embryos developing in vitro through the first phases of differentiation. The fertilized mouse eggs were taken from the oviducts at the two-four cell stage and grown in a nutrient medium at 37°C up to the hatching of the blastocysts after three days. The embryos were exposed to various doses of ^{3}H in different chemical forms, since the beginning of incubation.

A number of radiobiological end-points were considered but the block of cell divisions and the formation of abnormal blastocysts appeared as the most suitable parameters for evaluating radiation effects. Tritiated aminoacids incorporable into nuclear chromatin were as toxic for the mouse embryos as tritiated thymidine. These compounds exerted deleterious effects at concentrations of a few tens nCi/ml, or even less, in the incubation medium. The results indicate that ^{3}H decaying in or near the genetic material causes maximal biological damage.

B.8. Preparations of polydeoxynucleotides

The preparation of DNA-like polymers for assaying and characterizing enzymes active on DNA substrates and DNA templates was extended. The service function of supplying these polynucleotides to European laboratories working in the research areas of DNA damage and repair was continued. Synthetic polynucleotides or enzymes for their preparation were distributed to investigators at the Universities of Leiden, Rome, Brussels at Rhôde-St.-Génèse, Sussex and Pavia, and also to biophysicists from the University of Parma which were studying structural details of DNA pyrimidines at the 600 Mev syncrocyclotron facility for muon spin relaxation analysis of CERN in Geneva.

In collaboration with chemists from Leiden University, a new method for preparing solid-state polydeoxynucleotides was standardized. 5'-NH_2 terminated dT oligonucleotides were chemically synthesized and extensively elongated with dT sequences via the end-addition reaction catalyzed by TdT. The 5'-NH_2 poly(dT) products were coupled to CNBr-activated

cellulose and the resulting complexes served as grafting carriers to assemble DNA-like substrates immobilized on solid supports for more sensitive assays of nucleases and ligases.

DNA polymerase primers as well as DNase and DNA ligase substrates with molecular damage specifically located in portions of their nucleotides chain were also prepared.

A number of DNA template-initiator systems with mispaired radioactive terminal nucleotides were synthesized. These polymers had the general formula poly(dA):oligo(dT)-oligo($[^{14}C]$dC) and were used to characterize the 3'→5' "proofreading" exonuclease activity found in DNA polymerase preparation of mammalian origin.

Uracil-DNAs single and double stranded polydeoxynucleotides containing uracil in various proportions and partially apyrimidinic DNA-like polymers were prepared and employed to characterize uracil-DNA glycosylase and AP-endonuclease activities.

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III. 4.

SOMATISCHE KURZZEITWIRKUNGEN IONISIERENDER STRAHLEN (AKUTES STRAHLENSYNDROM UND SEINE BEHANDLUNG)

SOMATIC SHORT-TERM EFFECTS OF IONIZING RADIATIONS (ACUTE IRRADIATION SYNDROME AND ITS TREATMENT)

EFFETS SOMATIQUES A COURT TERME DES RAYONNEMENTS IONISANTS (SYNDROME AIGU D'IRRADIATION ET DE SON TRAITEMENT)

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Tätigkeitsbericht beschrieben : *

Further research work on these subjects will also be described in the following progress reports : *

D'autres travaux sur ce thème de recherche sont également décrits dans les rapports suivants : *

210-BIO D	Univ. Homburg (Muth/Grillmaier)
215-BIO D	KFA, Jülich (Feinendegen)
156-BIO B	Univ. Bruxelles (Brachet)
205-BIO D	GSF, Frankfurt (Pohlit)
232-BIO B	CEN, Mol (Maisin)
218-BIA D	GSF, Neuherberg (Gössner)
266-BIO UK	Univ. Oxford (Hopewell/Wiernik)
252-BIO UK	Univ. London (Lindop)
249-BIO UK	MRC, Harwell (Vennart)
256-BIO DK	Univ. Copenhagen (Danø)

* Siehe auch Punkt VI,
See also section IV,
Voir aussi point IV,

Contractant de la Commission : Georges Mathé, Directeur de l'Institut de Cancérologie et d'Immunogénétique (ICIG), Hôpital Paul Brousse, 14-16 avenue Paul-Vaillant-Couturier 94800 VILLEJUIF ; Association Claude-Bernard, 3 avenue Victoria 75004 PARIS

N° du contrat : 220-76-I-B10-F

Chef du(des) groupe(s) de recherche : Georges Mathé

Thème général du contrat : Protection against the pathological effects of high doses of irradiation by marrow grafting ; immunological and pathological studies of the graft-versus-host reaction provoked by incompatibility for minor antigens.

Titre du projet n°1 : Role of minor histocompatibility antigens in the development and the regulation of lethal graft-versus-host reaction (GVHR) developed across non-H-2 or H-2 barriers.

Chef du projet et collaborateurs scientifiques : Georges Mathé
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Marrow grafting could represent a valuable means of therapy for certain victims of heavy doses of irradiation as well as for cancer patients submitted to intensive thoracic radiotherapy or for leukemic patients conditioned for bone marrow grafting by lethal donor irradiation. The major obstacle currently encountered in this field by clinicians is that of mortality due to secondary disease, arising primarily as a result of the chronic graft-versus-host reaction (GVHR) caused by grafted cells. While development of a GVHR is conditioned mainly by antigens controlled by the major histocompatibility complex (MHC : HLA in Man and H-2 in Mouse) a lethal GVHR is often seen in recipients of MHC-identical marrow. This strongly suggested that minor histocompatibility antigens (MiHA ; i.e. those not controlled by the MHC) are important in the development of GVHR-related mortality.

In the present work we have studied the role of MiHA in lethal GVHR by using the Mouse as experimental model. Since mouse MHC is analogous to that of Man, both from a genetic and from a physico-chemical point of view, one may reasonably hope that a similar analogy exists for MiHA. Since 1976 we have studied the following points :

A) Role of MiHA in lethal GVHR developed across non-H-2 barrier

a) incompatibility for MiHA alone may induce a high rate of mortality i adult recipients of normal donor cells

b) interactions between MiHA and consequences of these interactions on lethal GVHR

i) cumulative effect of incompatibility for several MiHA detectable by lethal GVHR developed by backcross donors

ii) number of minor genes involved in lethal GVHR and interactions between MiHA defined by studying 10 genetically independent chromosome markers

B) Interactions of MiHA and H-2 antigens in lethal GVHR developed across H-2 barr

a) for a given H-2 haplotype the effects of MiHA and H-2 antigens may or may not be cumulative depending on the nature of the MiHA

b) for a given set of MiHA the effects of MiHA and H-2 antigens may be cumulative or "suppressive" depending on the H-2 haplotype.

A) Role of MiHA in lethal GVHR developed across non-H-2 barrier :

a) incompatibility for MiHA alone may induce a high rate of mortality in adult recipients of normal donor cells :

The notion that antigens controlled by the MHA are "all-important" for the development of lethal GVHR disease has permeated scientific thinking in this field, so that the effects of MiHA are often considered as relatively insignificar unless either neonatal hosts or immunized donor cells are used. Our results clearl show that incompatibility for MiHA alone may induce lethal GVHR in adult recipient of normal cells. However, the severity of the lethal GVHR developed across a non-H-2 barrier varies as a function of the different genetic combinations. For exampl it is relatively weak and slow after grafting of B10.BR ($H\text{-}2^k$) donor cells to (B10.BR x AKR)F1 ($H\text{-}2^k$) recipients (30% of mortality 100 days after grafting), but it is rapid and strong after grafting of B10.D2 ($H\text{-}2^d$) donor cells to (DBA/2 x B10.D2)F1 ($H\text{-}2^d$) recipients (98% of mortality 100 days after grafting). In this la series of experiments (DBA/2 x B10.D2)F1 mice were grafted with cells from the congenic resistant B10.D2 ($H\text{-}2^d$) strain, whose $H\text{-}2^d$ allele is presumably identical to that of the DBA/2 strain from which it is derived. Hence the GVHR which develop in this strain combination is clearly directed against DBA/2-MiHA (D2-MiHA) expressed in the recipient.

For this strong mortality to be induced, the presence of mature immunocompetent (spleen) cells in the graft inoculum is required : no mortality was observed after grafting of 10^7 B10.D2 bone marrow cells alone, although the presence of as few as 10^6 additional spleen cells permitted the induction of

mortality in 57% of grafted (DBA/2 x B10.D2)F1 recipients.

High doses irradiation of adult recipients increases their susceptibility to MiHA-related GVHR mortality : in F1 recipients irradiated at 300 or 500 rads no lethal GVHR could be observed, regardless of the cell suspension grafted. In contrast, when F1 recipients were irradiated at 900-1100 rads the grafting of 10^7 bone marrow and $8x10^6$ spleen cells resulted in death of 98% of recipients, while all F1 recipients grafted with isogenic cells survived indefinitely.

Since a parent-to-F1 donor-recipient combination was employed, it is unlikely that this phenomenon is related to a decreased host-versus-graft reaction subsequent to radiation-induced immunosuppression ; the increase of sensitivity to bacterial and/or viral infections combined with GVHR-induced immunosuppression could provide a possible explanation. This hypothesis is supported by the observation that very potent suppressor cells are present in the animals undergoing the lethal GVHR (unpublished). Furthermore, the importance of the microbiological environment of the animal colony is illustrated by the fact that MiHA-related GVHR mortality of lethally irradiated conventional recipients may be dramatically rapid (as rapid or even more rapid than mortality of irradiation controls), but that this acute GVHR mortality is considerably delayed by donor and recipient treatment with a series of large-spectrum antibiotics (unpublished).

Another, yet unclarified, observation is that none of mice receiving parental marrow and spleen cells in combination with isogeneic marrow died.

b) Interactions between D2-MiHA and consequences of these interactions on lethal GVHR :

i) cumulative effect of incompatibility for several MiHA detectable by lethal GVHR developed by backcross donors.

After observation that the difference for D2-MiHA alone may lead to a high rate of mortality of nearly 100% of the recipients, we asked next whether the effect of incompatibility for several DBA/2 minor loci is cumulative. We tried to answer this question by comparing the survival time in donor-recipient combinations incompatible for either all D2-MiHA or for a reduced number of D2-MiHA. To study the effect of all D2-MiHA, B10.D2 donor cells were grafted to (DBA/2 x B10.D2)F1 recipients. To study the effect of a reduced number of D2-MiHA the (DBA/2 x B10.D2)F1 mice were backcrossed to the B10.D2 parent strain. While these backcross mice inherited the entire B10.D2 genetic background, they inherited only a part of the DBA/2 background, hence only some of D2-MiHA. Consequently, when grafted to (DBA/2 x B10.D2)F1 recipients, cells from backcross donors may develop a GVHR only against those D2-MiHA which are absent from their own genetic background 120 backcross donors were studied individually ; the bone marrow and spleen cells from each backcross donor were grafted to three F1 recipients. The results showed

that under these genetic conditions, i.e., when the number of incompatible D2-MiHA is reduced, the median survival time is significantly prolonged and the percentage of surviving recipients is considerably increased. These results indicate that the effect of several D2-MiHA is cumulative and that the number of incompatible minor loci is an important factor in the lethal GVHR developed across a non-H-2 barrier. This result is important, since a cumulative effect of several MiHA was found in the skin graft rejection (Graff R. and Bailey D.W., 1977), but no equivalent studies were done in the GVHR. However, it may be envisaged that MiHA important in skin graft rejection or in GVHR are not necessarily the same, and certain of the following results favour this hypothesis.

ii) number of minor genes involved in lethal GVHR and interactions between D2-MiHA defined by studying 10 genetically independent chromosome markers.

While several MiHA of various antigenic strengths involved in skin graft rejection in mice are well-defined (R. Graff 1973) very few minor loci important in GVHR have been studied to date (Cantrell and Hildeman 1973). In one series of experiments in our laboratory, minor histocompatibility genes of DBA/2 strain, linked to ten genetic markers (Table I) were studied with respect to their influence on lethal GVHR. For this purpose the individuals from litters of (DBA/2 x B10.D2)F1 hybrids mice backcrossed to B10.D2 strain were used as donors, and (DBA/2 x B10.D2)F1 hybrid mice as recipients. 92 backcross donors were studied individually and bone marrow and spleen cells from each backcross donor were grafted to three F1 recipients. Each backcross donor was types for its genetic constitution for the ten genetically independent markers expressed in different allelic forms in B10.D2 and DBA/2 strains of mice. Since there is no marker yet known to be associated with genetic background region important in the lethal GVHR, the markers studied in the present work were chosen at random. The mortality induced by backcross donors either homozygous or heterozygous for a single marker were compared. Moreover, with the aim of demonstrating the existence of histocompatibility genes whose isolated effect is too weak to be detected, we looked for a possible cumulative effect between such genes. For this purpose, we studied the GVHR-mortality in relation to the allelic form of two markers taken together.

When each marker was studied independently, five markers appeared to be genetically linked to genes affecting lethal GVHR (Table II). Four of them, Id-1 (chromosome 1), Gpd-1 (chromosome 4), Gpi-1 (chromosome 7) and Es-3 (chromosome 11) were found to be linked to "classical" histocompatibility genes, i.e. genes capable of increasing the intensity of GVHR. In contrast, another gene, linked to the marker Pgm-1 (chromosome 5), was found to have a suppressive effect on GVHR mortality. No correlation was found with the Hbb marker. As indicated in Table II,

TABLE I : Chromosome markers studied

Chromosome assignment	Abbreviated symbol	Complete name of the marker	Allelic form in the background		Closest minor histocompatibility gene	Distance in crossing-over units	Reference of the technique used for detection
			B10.D2	DBA/2			
Ch.1	Id-1	Isocitrate dehydrogenase-1	a	b	-	-	HENDERSON, 1965
Ch.2	Svp-1	Seminal vesicle protein-1	b	a	H-13	12	MOUTIER et al., 1971
Ch.4	Gpd-1	Glucose phosphate dehydrogenase-1	a	b	H-20	1	RUDDLE et al , 1968
Ch.5	Pgm-1	Phosphoglucomutase-1	a	b	H-27	12	SHOWS et al., 1969
Ch 7	Hbb	Hemoglobin beta-chain	s	d	H-1	1	RUSSEL & BERNSTEIN, 1966
Ch.7	Gpi-1	Glucose phosphate isomerase-1	b	a	H-24	2	DE LORENZO & RUDDLE, 1969
Ch.8	Es-1	Serum esterase-1	a	b	H-29	17	POPP, 1968
Ch.9	Mod-1	Malic enzyme (supernatant)	b	a	H-7	12	SHOWS & RUDDLE, 1968
Ch.11	Es-3	Kidney esterase-3	a	c	-	-	RUDDLE, 1966
?	Svp-2	Seminal vesicle protein-2	a	c	-	-	MOUTIER et al., 1971

TABLE II

- Four "classical"-type minor histocompatibility genes (responsible for the increase of lethal GVHR) are mapped on the following chromosomes :

  1 (Id-1 marker - not linked to any minor histocompatibility gene known to be important in skin graft rejection).

  4 (Gpd-1 marker - linked to the H-20 minor histocompatibility gene known to be important in skin graft rejection).

  7 (Gpi-1 marker - linked to the H-24 minor histocompatibility gene known to be important in skin graft rejection).

  11 (Es-3 marker - not linked to any minor histocompatibility gene known to be important in skin graft rejection).

- A minor histocompatibility gene(s) having a suppressive effect (responsible for the decrease of the lethal GVHR) is mapped on chromosome :

  5 (Pgm-1 marker - not linked to any minor histocompatibility gene known to be important in skin graft rejection).

- No correlation was found between lethal GVHR and the Hbb marker (chromosome 7) closely linked to the H-1 minor histocompatibility gene, known to be important in skin graft rejection.

these results strongly suggest that the MiHA important in skin graft rejection and GVHR are not necessarily the same.

When the markers were studied in couples, all appeared to interact, affecting the severity of lethal GVHR. Since the markers studied were chosen at random and since each among them gave the information on the distance of only 5-10 crossover units on each side of the marker, these results suggest that the number of genes influencing GVHR through their interactions is very high (>100). The combined effects of genes linked to two different markers was found to be either cumulative, or synergistic or "suppressive". In this last case, the effect of relatively strong minor histocompatibility genes (i.e., a gene linked to the marker Gpi-1) may be masked by a suppressive effect of another minor gene (i.e., a gene linked to the marker Pgm-1) (Fig. 1). Moreover, the gene(s) linked to a single marker may also have opposing effects. As a consequence, the net effect may be nil when this gene is considered alone (e.g., gene(s) linked to the marker Mod-1). The interaction between the genes is highly specific ; i.e., the effect of suppressor genes linked to Pgm-1 is very efficient for the histocompatibility gene linked to Gpi-1 while it exerts a synergistic effect with a gene linked to the marker Es-3.

In conclusion, the results taken together indicate that the genetic background is very important for the development of lethal GVHR. The effect of the minor genes analyzed individually is weak, and the number of genes capable of influencing GVHR mortality individually is relatively low as compared to the number of genes influencing lethal GVHR through their interactions. The interactions between these genes are specific, complex and strong. However, the effects of genes linked to two different makers, and even of those linked to a single marker, may be antagonistic and reciprocally neutralized. This observation may partially explain why in some genetic combinations the incompatibility for MiHA appears as relatively unimportant. These results show for the first time that the intensity of a GVHR developed against MiHA is dependent not only on the number of incompatible MiHA and allelic combinations involved, but that it is also highly dependent on the interactions between these MiHA which effects may be very different. Finally, the minor histocompatibility antigens important in organ and bone marrow grafting are not necessarily the same.

B) Role of MiHA in lethal GVHR developed across H-2 barrier :

While the effects of incompatibility involving several MiHA plus H-2 antigens have been extensively studied in the skin graft model and have been found in some circumstances to be cumulative (R.J. Graff 1973), there is little if any information on the combined effects of MiHA and H-2 antigens in the GVH

reaction. Our results show that the effects of MiHA and H-2 may, in some cases, be cumulative, but that these effects vary depending on : a) the nature of the MiHA involved, as well as on b) the H-2 haplotype with which a given set of MiHA are associated ; and that in some genetic combinations the response to MiHA plus H-2 antigens, instead of being stronger, may be weaker than the response to H-2 antigens alone.

a) for a given H-2 haplotype the effects of MiHA and H-2 antigens may or may not be cumulative depending on the nature of the MiHA.

In lethal GVHR induced by grafting B10 ($H\text{-}2^b$) donor cells the mortality of the (DBA/2 x B10)F1 ($H\text{-}2^{d/b}$) recipients incompatible for D2-MiHA and H-2 is significantly more rapid than that of the (B10.D2 x B10)F1 recipients incompatible for $H\text{-}2^d$ alone. In contrast, the mortality of the (Balb/c x B10)F1 ($H\text{-}2^{d/b}$) recipients incompatible for Balb/c-MiHA and $H\text{-}2^d$ is identical to that of the (B10.D2 x B10)F1 recipients incompatible for $H\text{-}2^d$ alone. Thus, in lethal GVHR induced by grafting the same $H\text{-}2^b$ donor cells, the effects of $H\text{-}2^d$ antigens and D2-MiHA were cumulative while no cumulative effect was found for $H\text{-}2^d$ antigens and Balb/c-MiHA ; this indicates that the combined effects of MiHA and H-2 antigens may be cumulative, and that for a given H-2 haplotype, a cumulative effect may or may not be detected depending on the nature of these MiHA.

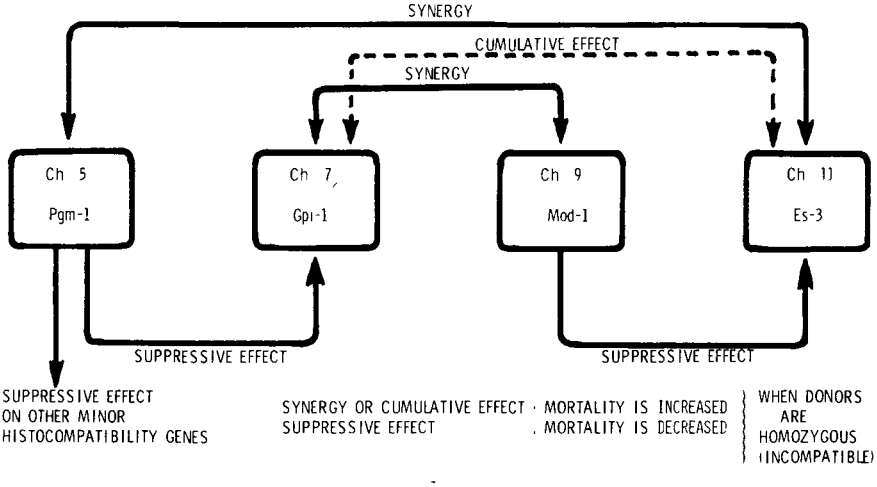
It should be emphasized that the cumulative effect of D2-MiHA and $H\text{-}2^d$ antigens was not found when the intensity of GVHR was measured by splenomegaly test, and that it was shown not to be related to the genetic resistance of the recipient.

b) for a given set of MiHA the effects of MiHA and H-2 antigens may be cumulative or "suppressive", depending on the H-2 haplotype.

In next series of experiments we studied whether the effect of the same (D2) MiHA can vary as a function of the H-2 haplotype with which they are associated ; we compared the mortality induced after grafting the same parent-strain (B10.D2) donor cells to various F1 recipients incompatible for : 1) D2-MiHA alone ((DBA/2 x B10.D2)F1), 2) H-2 (k or b) antigens alone ((B10.D2 x B10.BR)F1 or (B10.D2 x B10)F1), 3) or D2-MiHA plus H-2 (k or b) antigens ((DBA/2 x B10.BR)F1 or (DBA/2 x B10)F1). The results show that the lethal GVHR developed to D2-MiHA plus $H\text{-}2^k$ antigens is more severe than that developed to $H\text{-}2^k$ alone, while the lethal GVHR developed to D2-MiHA plus $H\text{-}2^b$ antigens is, surprisingly <u>less</u> severe than that developed to $H\text{-}2^b$ antigens alone. Thus, the effect of an additional incompatibility for a given set of MiHA may be

cumulative or "suppressive" depending on tne H-2 haplotype with which these MiHA are associated. This strongly suggests that, in the DBA/2 genetic background, there are minor genes (such as those linked to the Pgm-1 or Mod-1 markers ?) capable of activating an immune process which has a suppressive effect on the anti-H-2 response ; this suppressive effect is or is not detectable depending on the H-2 haplotype. Variations in the intensity and/or the kinetics of the response to different H-2 haplotypes and relation to the cumulative versus independent gene effect (D.W. Bailey, 1971, 1980) may, at least partially, explain these results.

INTERACTIONS BETWEEN MINOR HISTOCOMPATIBILITY GENES LINKED TO THE GENETIC MARKERS Pgm-1, Gpi-1, Mod-1 AND Es-3 IN LETHAL GVHR



Titre du projet n°2 : Histopathologic sequence of events in adult mice undergoing lethal graft-versus-host reaction developed across H-2 and/or non-H-2 histocompatibility barriers.

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The sequence of histologic events in graft-versus-host reaction (GVHR) caused by major and/or minor histoincompatibilities was studied. The effect of major histoincompatibility (involving the difference for H-2 antigens and minor histocompatibility antigens (MiHA)) was studied in (DBA/2 x B10)F1 recipients after grafting of B10 ($H\text{-}2^b$; B10-MiHA) normal donor cells. The effect of minor histoincompatibility (involving the differences for MiHA alone) was studied in (DBA/2 x B10.D2)F1 recipients after grafting of B10.D2 ($H\text{-}2^d$; B10-MiHA) normal donor cells. In both situations the recipients were adult mice irradiated at 1100 rads.

Our study demonstrates that the pathologic changes in GVHR vary, depending on the degree of histoincompatibility between donor and recipient. We were able to recognize two graft-versus-host disease entities, each with its own clinical presentation and relatively distinctive pathologic findings. Both progress in stages and with varying rapidity. The disease caused by major histoincompatibility (H-2 and non-H-2 barrier) justifies the use of the term acute or "major" GVHR, and the one caused by minor histoincompatibility (non-H-2 barrier alone), the term chronic or "minor" GVHR.

In the "major GVHR", four phases were recognized : a) transien aplasia, b) repopulation, c) a proliferative, invasive phase, or phase of emigration, and d) a terminal phase of acute organ rejection. Major GVHR runs a short course.

The stage of transient aplasia following irradiation lasts from about 24 hours to 4 days. In this stage, the external appearance of the animal was normal. Pathologically, bone marrow aplasia and depletion of small lymphocytes in the thymus, splenic white pulp, lymph nodes, and Peyer's patches were observed.

The stage of cellular repopulation started between the 4th and 7th days after irradiation. Clinically, the animals were free of symptoms and signs of disease. Histologically, granulocytic hyperplasia was seen in the bone

marrow and in the red pulp of the spleen. Lymphoblastoid cells were observed in the thymus, lymph nodes, and Peyer's patches.

The proliferative, invasive phase began by Day 7. The animal appeared ill, the fur became dull, and diarrhea began to appear. Microscopically, the lymphocytes proliferated and invaded the tissues. The hepatocytes showed early vacuolar changes. The skin became atrophic. Lymphoblastoid cellular infiltrations were seen in the lymph nodes and spleen.

The stage of acute organ rejection was seen by Day 11. The animals showed evidence of weight loss, and the diarrhea increased. Microscopically, the liver cells became more vacuolated. The polymorphonuclear leukocytes started adhering to the vascular endothelium of the lungs, kidney and liver. Large lymphoblastoid cells invaded the organs. The thymic epithelium became atrophic, and the thymus was completely devoid of small lymphocytes. Fragmentation of reticular fibers and necrosis were prominent in the paracortical zone of the lymph node. Cardiac, renal, interstitial, and hepatic signs described in animals with acute organ rejection (Marceau et al., 1965 ; Williams et al., 1968 ; Hume, 1971 ; Jerusalem and Jap, 1977) were observed. The vacuolization of the tubular epithelium of the kidney early in the disease may be attributable to hypokalemic nephrosis secondary to intestinal lesions or may be the result of direct immunologic injury of the tubular cells. This phase was terminal. The cause of death was organ failure secondary to rejection by the grafted cells. The adhesion of the polymorphonuclear leukocytes to the vascular endothelium represented good evidence for organ rejection (Hume 1971 ; Jerusalem and Jap, 1977). The polymorphonuclear leukocytes, lymphoblastoid cells, and macrophages seemed to be the cellular elements which play the major role in this disease entity. The thymic epithelial degeneration was striking, and it started earlier than in the chronic "minor" type of GVHR.

In the "minor GVHR", we recognized six phases : a) transient aplasia, b) repopulation, c) lymphoid proliferation manifested by early tissue infiltration with lymphocytes presumed to be immunocompetent cells, d) a phase of major immunologic tissue injuries, e) a phase of repair, and f) a terminal phase of advanced sclerosis in which scarring of the injured tissue was occasionally associated with epithelial hyperplasia.

No marked differences were observed between the first two phases of the "major" and the "minor" GVHR disease. It was not until the third (proliferati

phase that the histopathologic features of minor GVHR became distinctive. By Day 7, lymphoid cells, presumably of donor origin, appeared in the vascular channels and infiltrated the organs where they exhibited mitotic activity. Clinically, skin eruptions were seen. Histologically, most of the lymphoid proliferations were seen in the T-cell areas of the lymphoid organs. The small vessels and capillaries were packed with lymphocytes. In this phase, early degenerative changes were seen in the thymic epithelium, although it was still intact. Collections of 10 to 12 lymphoid cells were seen in the perivascular areas of the kidney and in portal tract of the liver.

Major immunologic injuries (fourth phase) became apparent by Day 15. The early injuries consisted of degeneration of the collagen in the dermis, muscular fascia, adipose tissue, vessel walls, and paracortical areas of the lymph nodes. This degeneration progressed to necrosis by Day 18. Destruction of lymphocytes was also evident. Between Days 18 and 25, the epithelial cells of the thymus completely disappeared ; this crucial event was followed by marked plasma cell proliferation in the bone marrow. During this period, proliferation of macrophages and early fibrosis heralded the phase of repair (fifth phase). Venous thrombi were evident in the cutaneous and portal veins. The kidney showed chronic progressive glomerulonephritis. The major causes of death in this phase were acute heart failure or pulmonary infections. A striking feature during the phase of repair was the granulomatous panniculitis. which contributed to the pronounced thickening of the subcutis and coincided with the animals' weight gain. We are not aware of reports describing a weight gain of this magnitude due primarily to granulomatous panniculitis in chronic GVHR of experimental animals, although chronic panniculitis has been mentioned in some reports of chronic GVHR in animals (Van Bekkum and de Vries, 1967 ; Hobik, 1977) and man (Slavin and Santos, 1973).

In the terminal (sixth) phase of advanced sclerosis, the skin showed marked scleroderma, alopecia, and keratotic plugs in the hair follicles. Russell bodies were seen in the red pulp of the spleen. Splenic infarctions were evident. Bone marrow fibrosis was advanced. Arteriolosclerotic changes were observed in the kidneys. Glomerulosclerosis was a constant finding. The massive lymphoblastoid proliferations in the splenic red pulp and the leukoplakia of the esophagus were considered to be hyperplastic and possibly premalignant changes.

Atrophy of the thymic epithelium was first observed on Day 11 and became more pronounced by Day 15. On day 18, the thymic epithelial cells showed severe degeneration. Beginning with Day 18, when all of the animals with "major" GVHR had died, the proliferation of plasma cells in the animals with "minor" GVHR rapidly increased over the next 7 days, localizing at sites where they normally are not present (thymus and T-cell areas of spleen and lymph nodes). Russell bodies were abundant, indicating active antibody production by the proliferating plasma cells. This apparently uncontrolled B-cell proliferation suggests that the corresponding suppressor T-cell activity was greatly diminished. The fact that this coincided with atrophy of the thymic epithelium is consistent with the hypothesis that the intact thymus is necessary for the induction of suppressor cells (Reinisch et al., 1977). Thus, destruction of the thymic epithelium may have resulted in a diminution of suppressor T-cells and consequently in an unopposed proliferation of plasma cells. The role of injury to the thymic epithelium in the pathogenesis of GVHR is also suggested by the rapid onset of GVHR induced by grafting of bone marrow cells in patients with thymic dysplasia (Miller, 1967 ; Miller and Hummeler, 1967).

In the present work, lesions caused by GVHR were specifically due to immunological injuries, as has been widely recognized. (Van Bekkum and de Vries, 1967 ; Hobik, 1977 ; Slavin and Santos, 1973 ; Mathé, 1961 ; Krüger et al., 1971 ; Grundmann and Hobik, 1973). Absence of similar lesions in the isologous group proves the immunologic nature of the changes described in both major and minor GVHR disease.

Titre du projet n°3 : Attempt to prevent lethal GVHR developed across non-H-2 or H-2 barriers.

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A) Attempt to prevent lethal GVHR developed across non-H-2 barrier :

In some genetic combinations, the grafting of donor cells incompatible for MiHA alone may lead to a strong GVHR and kill a high percentage of recipients. For example, after grafting of B10.D2 cells (H-2^d) to the (DBA/2 x B10.D2)F1 recipients incompatible for DBA/2 (D2)-MiHA alone only 2% of recipients survive. Preimmunization of the B10.D2 donor cells with specific (D2-MiHA) and/or nonspecific (H-2^b) histocompatibility antigens was performed and it was found to have the following effects on lethal GVHR :

- mortality identical to that induced by normal cells (no effect) ;
- mortality accelerated as compared to that induced by normal cells ;
- mortality delayed and/or decreased as compared to that induced by normal cells.

The effect of donor immunization is highly dependent upon the conditions used for the immunization (inverval between day of immunization and day of grafting, dose of antigen, the physical form in which the antigens are presented, i.e. whole cells versus cell extracts).

Acceleration of mortality is favoured by multiple injections of the antigens, a long interval between the day of immunization and the day of grafting, and (preliminary results) by increased doses of specific antigens.

Survival is improved by a single immunization a short time before grafting and (preliminary results) by increased doses of nonspecific antigens.

The most efficient reduction of mortality is observed after donor immunization with a single injection of specific D2-MiHA, administered alone or in association with unrelated H-2^b antigens a short time before grafting. The percent of surviving recipients is considerably greater after donor immunization with specific D2-MiHA alone (80% of recipients survive) than after immunization with nonspecific H-2^b antigens alone (27% of recipients survive). However, the percent of surviving recipients is increased even more when the donors are immunized simultaneously with specific D2-MiHA plus unrelated H-2 antigens (93% of recipients survive) ; this suggests

that the "suppressive" effect induced by association of these two types of immunization is cumulative ; and that donor immunization with unrelated H-2 antigens alone may (although slightly) decrease the GVHR mortality developed by incompatibility for MiHA alone. The beneficial effect of donor immunization with unrelated H-2 antigens is illustrated by four additional findings :

- early mortalities (10 days after grafting) may be observed after donor immunization with either specific D2-MiHA or unrelated H-2^b antigens alone (about 10%) but never after combined immunization with D2-MiHA plus H-2^b.
- Under certain experimental conditions inducing a secondary response to D2-MiHA (in heavily irradiated primary recipients, Cerottini 1971), this secondary response is suppressed when donor cells are pre-immunized simultaneously against specific D2-MiHA plus unrelated H-2^b or H-2^k antigens.
- Mortality may be delayed by donor treatment with the unrelated H-2^b lipoprotein extract.
- Suppressor cells detectable by an in vitro test (MLR) were found after three types of immunization (specific, nonspecific or specific plus nonspecific) but they were more efficient after immunization with specific D2-MiHA plus nonspecific H-2^b than after immunization with either specific D2-MiHA or nonspecific H-2^b antigens alone.

B) Attempt to prevent lethal GVHR developed across H-2 barrier :

a) Decrease of GVHR mortality by the serum from donors treated with recipient-specific soluble histocompatibility antigens ; mechanism of action.

Cellular and humoral immunity were studied in C57BL/6 (H-2^b) mice after treatment with allogeneic DBA/2 (H-2^d) soluble histocompatibility antigens in the conditions previously shown to decrease specifically the host-versus-graft reaction. Cellular immunity was found to be diminished as measured by mixed lymphocyte reaction (MLR) and GVHR, but more frequently when measured by GVHR related mortality than when measured by GVHR related splenomegaly developed in (DBA/2 x C57BL/6)F1 mice. Serum from treated mice is antibody-free ; but it enhances skin allograft survival if transferred to normal hosts, diminishes the reactivity

of normal cells when present during MLR or when incubated with the responding cells prior to MLR, and delays GVHR mortality when incubated with donor cells prior to grafting. The blocking activity of the serum is "dose"-dependent : the in vivo blocking effect of the undiluted serum depends on the dose of soluble antigen used for its production, and the in vitro blocking effect of the serum (raised by the same dose of soluble antigen) depends on its concentration during MLR. Three different serum-concentration-dependent effects were found in MLR experiments : an inhibition with high and a stimulation with intermediate serum concentrations. Similar results were observed with either antibody-free serum (raised by soluble antigens) or "classical" hemagglutinating enhancing sera (raised by injection of lyophilised spleen cells). It was concluded that the decreased cellular immunity of soluble antigen-treated mice is related to a serum blocking factor, interacting with T cells, which is not an antibody but rather another type of suppressor factor (soluble antigen or an antigen-antibody complex or suppressor factor released by suppressor cells). Whether the T cell will distinguish the signal as being "immunogenic" or "tolerogenic" apparently depends on the level of the serum blocking factor. The T cell blocking activity of soluble antigen-raised and classical enhancing sera seems to be due to the same or a similar factor ; thus the immunosuppressive activity of classical hemagglutinating enhancing sera may not be entirely dependent on free antibodies.

b) Decrease of GVHR mortality by specific H-2 antigens and nonspecific MiHA : lipoprotein preparations.

The pretreatment of C57BL/6 ($H\text{-}2^b$; B6-MiHA) donors with Balb/c ($H\text{-}2^d$; Balb/c-MiHA) lipoprotein preparation delayed mortality in (C57BL/6 x DBA/2)F1 recipients more efficiently than the pretreatment with DBA/2 ($H\text{-}2^d$; specific D2-MiHA) lipoprotein preparation. This indicates that for maximum effectiveness the extract used for donor treatment should contain MiHA that differ from those present in the GVHR target. These results taken together with those obtained in the experiment on GVHR mortality developed across non-H-2 barrier (see chapter III.A) strongly suggest that in both situations (H-2 and non-H-2 barriers) the GVHR mortality is more efficiently suppressed by donor immunization with specific plus nonspecific histocompatibility antigens than by donor immunization with specific histocompatibility antigens alone.

c) Prevention of graft-versus-host reaction (GVHR) by thymic and splenic factors.

Parental spleen cells were preincubated with thymic and splenic non-species-specific extracts before grafting to sublethally irradiated hybrid recipients. By this procedure GVHR has been avoided. Furthermore, these extracts are devoid of any toxicity or proliferation inhibitory effects on grafted stem cells. The thymic extract specifically inhibiting T lymphocyte proliferation was purified by fractionated precipitations with ethanol followed by either filtration through Sephadex column and ion exchange chromatography, or ammonium sulfate precipitation and preparative electrofocussing. Two active fractions have been isolated, the more purified one, precipitated in a pH zone 6 - 6.3, exhibited one main and 3-4 minor bands when submitted to analytical electrophoresis. An active peptide (SDIP : spleen derived immunosuppressive peptide) was isolated from the crude splenic extract by Dr. M. Lenfant (Poitiers). We showed that this highly purified (107-fold) peptide is capable of preventing GVHR at very low concentrations (pg ml^{-1}).

C) Control of GVHR and autorestoration of granulocyte precursors in irradiated mice pretreated with Corynebacterium parvum or BCG :

Corynebacterium parvum (CP) has been described, on the one hand, as an immunodepressor of cell-mediated immunity, even when administered at doses which stimulate other components of the immune machinery, and, on the other hand, as an agent capable of abrogating the genetic resistance of some strains of mice to hemopoietic transplants. We have therefore studied the effect of CP on the graft-versus-host reaction (GVHR), which is an immune reaction mediated by T lymphocytes, and which may be susceptible to genetic resistance in the strain combinations we utilize.

GVHR was induced in adult (C57BL/6 x DBA/2)F1 mice, sublethally irradiated and grafted with $2x10^7$ C57BL/6 spleen cells, and the mortality of these mice was recorded. Mortality was delayed when either donors or recipients or both were pretreated with 1.5 mg of CP 7 days prior to cell transfer. Combined treatment of both donors and recipients resulted in better survival than did treatment of donors or recipients alone.

On the other hand, in an experiment where BCG was used to prepare mice of several strains for an allogeneic marrow graft, BCG enhances survival in a

group of mice later found to be autorestored. For this reason we tested the effects of BCG and of Corynebacterium parvum on endogenous spleen colonies found in heavily irradiated mice. When (C57BL/6 x DBA/2)F1, C57BL/6 and DBA/2 mice were irradiated at 800 rads (the LD 100's of these strains being somewhat higher) eight days after irradiation significant numbers of endogenous colonies were detected in their spleens only if they had been pretreated with 1 mg BCG (i.v. on day -14) or 1.5 mg Corynebacterium parvum (i.v. on day -7). The number of colonies seen in a given strain was the same for both agents, although those developing after Corynebacterium parvum treatment were visibly much larger than those developing after BCG treatment ; but different numbers of colonies were observed in the different strains. Histological examination of the spleens of these animals revealed that 100% of the endogenous colonies developing in pretreated animals were granulocyte colonies, indicating a stimulation of granulocyte precursor replication and/or differentiation by the agents studied. Thus it is clear that such pretreatment can stimulate myeloid autorestoration under certain circumstances. At a higher irradiation dose fewer colonies were observed, suggesting that the dose of irradiation employed in preparing a recipient for grafting is extremely critical in determining whether autorestoration will occur.

Titre du projet n°4 : Role of recipient and donor sex in graft-versus-host reaction (GVHR).

Chef du projet : Georges Mathé
Collaborateurs scientifiques : Linda L. PRITCHARD, Basile TSOUCANAS

The role of recipient and donor sex in the GVHR developed subsequent to bone marrow grafting in irradiated mice was studied. Preliminary results indicated that, under certain experimental conditions, F1 female recipients of sex-matched parent-strain lymphoid cells seem to be considerably more resistant to the effects of a GVHR (as measured by mortality) than are male recipients. Since the experimental models used were ones in which genetic resistance of the recipients to the graft of parent-strain hematopoietic and lymphoid cells is known to be quite intense (C57BL/6 →(C57BL/6 x DBA/2)F1 or (C57BL/6 x Balb/c)F1), the relative intensities of genetic resistance in males and females of the two recipient strains were also estimated, in an effort to determine whether variations in genetic resistance alone might not account for the differences in mortality seen in male and female F1 mice undergoing a systemic GVHR. Finally, in order to explore the possibility that sex-related hormones might influence GVHR severity, donors and/or recipients in one series of experiments were castrated or sham-operated at least four weeks before bone marrow grafting.

The results obtained indicate that : (1) Marrow graft "take" is slightly better when donor and recipient are sex-matched than when they are sex-mismatched. (2) When measured by the CFU-s assay for hemopoietic stem cells, the genetic resistance of female F1 mice to B6 cell grafts is more intense than is that of male mice. (3) There is no difference between sexes in the intensity of genetic resistance to a lymphoid leukemia (EAkR) of B6 origin. (4) In sex-mismatched donor-host strain combinations, the H-Y antigen may influence graft rejection or GVHR intensity in some systems, while being without effect in others. (5) Spleen cells from age-matched male and female donors exhibit similar activities in mixed lymphocyte culture in vitro. (6) Donor castration (whether of males or of females) has no influence on the capacity of donor marrow to restore hemopoiesis or to induce a lethal GVHR. (7) Castration of male recipients, while having no significant influence on GVHR mortality after a sex-matched marrow graft, nevertheless seems to reduce their susceptibility to the GVHR induced by sex-mismatched (female) semi-allogeneic marrow cells, raising the possibility that the presence of male hormone(s) may exacerbate the female-anti-male GVHR.

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Titre du projet n° 5

BONE MARROW TRANSPLANTATION IN IRRADIATED SUBJECTS

Chef du projet et collaborateurs scientifiques : Georges MATHE et Léon SCHWARZENBERG.

Bone marrow transplantation stimulated great hope for treatment of aplasias and leukemias in 1958 following our first success in grafting this tissue. These trials, carried out from 1958 to 1963, proved that a permanent bone marrow graft from an allogeneic donor to a nonidentical twin recipient is possible, but it is often complicated by a so-called"graft-versus-host reaction" (GVHR) that we have observed and described since our very first trials in leukemic subjects.

This secondary disease immediately appeared as a formidable stumbling block to large scale application of bone marrow grafts in man. The description of antigens recognized by serum antibodies as shown by Bach and of antigens determined by the blastic transformation of lymphocytes in vitro (MLC = mixed lymphocyte culture) enabled us to determine a condition in which the graft acceptance is especially common : that in which donor and recipient are siblings and share the same antigens localised at the (HLA-A,B,C) and MLC major histocompatibility complex (MHC). Unfortunately, the chance of a recipient having a compatible donor ("matched" donor) in his family is only one in four, and with these conditions bone marrow grafts are further complicated by versus-host disease in one of two cases. This histocompatibility is thus only apparent in the tests that can determine the MHC ; it is not apparent in vivo where the minor histocompatibility antigens play an important role not only in mice but also in men.

Preparation of Recipient

In transplantation between identical twins no pre-transplant treatment is necessary. The recipient may, however, be given cytotoxic drugs in cases of leukemia, or immunosuppressive treatment if an "auto-immune" mechanism is suspected to be the cause of an aplasia.

In nonidentical twin donors who are MHC-compatible, a strongly immunosuppressive preparation is necessary and the best consists of total body irradiation with 1000 rads. The addition of a high dose of cytotoxic drug : either the cyclophosphamide propose by Santos can be used with success for leukemic patients.

The Graft

When the bone-marrow cells are grafted a more or less rapid restoration of the blood previously cytopenic occurs. Many tests provide evidence that the graft has taken. The frequency of graft take varies with the mode of conditioning and with histocompatibility : it is about 80 per cent when cytotoxic agents have been used and the donor-recipient pair is HL-A, MLC-compatible.

Direct and Indirect Evidence for Take of Graft

1. Direct evidence for the graft take

   1.1. Appearance in the blood of :

      1,11. Erythrocyte antigens of the donor

      1,12. HL-A antigens of the donor

      1,13. Granulocytic markers of the donor

      1,14. Sex chromosomes of the donor

   1.2. Appearance in the serum of donor's Allotypic immunoglobulin

   1.3. Appearance in the bone marrow of donor's sex chromosomes

2. Indirect evidence for the graft take

   2.1. Specific tolerance of donor's tissue Linked to the chimerism : skin graft

   2.2. Secondary disease linked to the graft-versus-Host reaction.

Graft-Versus Host Disease :
Prevention and Treatment

GVH disease has been observed at the time of our first transplantation and we have described its multiple manifestations : cutaneous (erythrodermia), digestive (diarrhea, "liver failure"), infectious (fever, septicemias), general (weight loss, asthenia), and hematologic (lymphopenia). Lymphopenia is not only

an expression of the disease, but of its major physiopathologic process, as it leads to an immune insufficiency responsible for infections that are usually extremely serious, being more often viral than bacterial. Lymphopenia is due to the destruction of the lymphocytes produced by the graft or is induced by them during their conflict with the recipient's antigens (GVH). This conflict explains the intestinal, hepatic, and cutaneous lesions that may, moreover, be made worse by infection. The intensity of the graft-versus-host-reaction may be slight, moderate, or severe.

The frequency of the GVH disease depends on various factors, especially the histocompatibility relationship and the pregraft preparation. Though not seen after grafts between monozygotic twins, it is the major complication in 70 per cent of matched transplants between siblings and in nearly 100 per cent of mismatched transplants.

Preventive treatment of disease generally consists of administration of methotrexate or cyclophosphamide after transplantation. These drugs were experimentally proposed by Uphoff and Santos et al. The suppression of the graft lymphocytes by density gradient sedimentation and their incubation in the presence of lymphocytic chalone have been proposed by Dicke et al. and by the present authors respectively.

Once the secondary disease is established, the administration of antilymphocytic globulin was the only method able in some cases to attenuate its manifestations. A curative effect of the serum has been on the other hand described in some occasions (Storb).

In fact, even in case of genotypically HLA compatible donors, secondary disease by GVH reaction can occur in 70 % of cases with a lethal risk in 20 %, a reaction that we have shown experimentally in congenic strains.

Therefore, a risk of disease does exist, even when the recipient possesses a genotypically compatible sibling donor.

The knowledge of factors for graft success are :

1. The good conditioning of the recipient by total body irradiation (TBI) used alone (in aplastic patiens) or associated to hight doses of cyclophosphamide (in leukemic patients).

2. The good status of the recipient at the time of transplant: aplastic patients at the beginning of their disease ; leukemic patients in remission of their disease .

3. The number of transplanted cells (which should be equal to or greater than 2.5 x 10^8 cells/kg) ;

4. The non-sensitization of the recipient in regard to the donor, by previous transfusions : under such conditions the rate of long survivals (probable cures) is 15/17 in the cases of aplasias.

This data leads us to the indications of more frequent bone marrow grafts, and which can be summarized as follows :

1. Aplasias with severe prognosis and before sensitization by transfusions.

2. Leukemias in remission : acute lymphoid leukemia in second remission : acute myeloid leukemia and blastic crisis of chronic myeloid leukemia in second or first remission, and before sensitization by transfusions.

3. Cases in which the recipients do not have genotypically competent donors.

In such cases, grafts, after conditioning by irradiation or cytostatics, have failed. On the contrary, if conditioning by antilymphocyte serum (ALS) which induce partial and transitory chimerism without GVH reaction, as shown by us since 1970, does not cure leukemia, it has nevertheless enabled us to obtain 4 survivals out of 24 patients with acute aplasia. This result has been confirme by Speck and Gluckman. It is possible that this result was not due to the graft, but to the effect of ALS on some physiopathologic mechanism of the aplasia (suppressive cells ?). However, our patient who have survived after 8 years are 4 subjects out of 5 in whom a transitory graft was obtained, whereas all the other patients in whom no graft was observed have died.

The treatment trials using antilymphocyte serum in aplastic patients who do not have genotypically identical donors, deserve to be continued.

In the last year (1980) 5 patients have been submitted to a bone marrow graft : 1 patient with aplastic anemia, 2 patients with acute myeloid leukemia, 2 patients at the beginning of a blastic crisis of chronic myeloid leukemia.

The patient with aplastic anemia is a young girl aged 16 who received marrow from his brother aged 10, she presented in months before grafting meningeal and plural haemorrhagias. She is one year later in perfect condition and their blood presents the figure of a chimera.

One AML patient lived 6 months in complete remission after the transplant but died of a liver failure due to GVH reaction. An other AML patient, in which since the beginning of the disease (one year former) a remission could not be obtained, lived 3 months in complete remission with normal haematology but died of relapse of the leukemia. One patient at the beginning of a blastic crisis of CML, grafted with the sister's marrow is in very good hematological status one year later (with blood chimerism) but presenting a GVH cutaneous reaction (lichenification of the skin in some places) controlled by prednisone from time to time. A second patient with CML died 2 months after grafting from an acute secondary disease.

These results indicate that : 1) in 5 cases out of 5, we have been able to obtain a success of the graft ; 2) in 3 cases out of 5 we have seen a GVH reaction ; 3) in 2 patients without GVHR, one is living in very good status ; the other died of a recurrence of the leukemia (the graft-versus-leukemia (GVL) reaction did not occur.

Our main project is now, as far as we seem to be able to obtain a graft in the great majority of cases, to try to prevent and control the graft-versus-host reaction, and especially to look at the role of so-called "minor histocompatibility antigens" in which our laboratory (Olga Halle-Pannenko, Georges Mathé) is making some good progress.

Titre du projet n°6 : Suppressor cells and GVH

Chef du projet et collaborateurs scientifiques : Georges MATHE and Simone ORBACH

Suppression of T-cell responses to histocompatibility antigens by BCG pre-treatment

Introduction :

The injection of high doses of BCG into mice is followed 2 weeks later by a decrease in the mitogen reactivity of their spleen cells. We considered the possibility that such a protocol could also induce a diminution in the splenic reactivity against transplantation antigens.

This hypothesis was tested in vitro in mixed lymphocyte reactions, and in vivo in graft-versus-host reactions.

Results :

- Effect of a previous intravenous BCG injection on spleen cell responsiveness in mixed lymphocyte reactions.

Three milligrams of BCG were injected intravenously into C57BL/6 mice, 14 da before the experiment, then spleen cells from normal or BCG treated mice were cultivated in vitro in presence of irradiated or non-irradiated DBA/2 spleen cells.

The response of the C57BL/6 spleen cells against the DBA/2 antigens in one way reactions are presented in Table 1. A striking decrease of the reactivity of BCG-treated C57BL/6 was observed.

When normal C57BL/6 spleen cells were cultivated with non-irradiated DBA/2 spleen cells in two-way reactions, the (^{3}H)-thymidine incorporation was nearly the double of that when cultivated with irradiated DBA/2 cells. The two-way reactions were almost entirely abolished when the C57BL/6 cells were obtained from BCG-treated mice.

T-cell populations from normal C57BL/6 mice were very active against DBA/2 (Table 2). However, a less striking reduction of the reactivity was observed when T-BCG cells were cultivated with DBA/2 spleen cells and when mixed with normal C57BL/6 (Table 2).

In contrast, normal C57BL/6-adherent cells responded weakly to DBA/2 (Table 2) and when BCG-adherent cells were cultivated with normal C57BL/6 and DBA/2 spleen cells, the reactivity was entirely abolished (Table 3).

Table 1. Suppressive activity of spleen cells from C57BL/6 mice 14 days after the injection of 3 mg BCG

C57BL/6 spleen cells	Number of cells per well	cultivated			suppression (%)	
		alone	+ irradiated DBA/2	non-irradiated DBA/2	irradiated DBA/2	non-irradiated DBA/2
Normal	$2.5x10^5$	25,424 ± 2,111	50,894 ± 10,608	114,916 ± 4,228	-	-
	$5.0x10^5$	45,172 ± 5,976	102,304 ± 4,761	119,876 ± 14,560		
BCG	$2.5x10^5$	4,873 ± 284	4,702 ± 393	5,637 ± 32	-	-
	$5.0x10^5$	3,707 ± 428.	5,201 ± 2,027	7,923 ± 675		
Normal + BCG	$2.5x10^5$ $2.5x10^5$	15,148 ± 1,768	22,599 ± 2,630	14,725 ± 2,331	7	100
Normal + BCG	$5.0x10^5$ $5.0x10^5$	24,439 ± 1,054	15,835 ± 3,260	15,207 ± 2,158	100	100
Normal + BCG	$2.5x10^5$ $5.0x10^5$	14,565 ± 794	15,774 ± 4,206	11,868 ± 645	95	100

Table 2 Suppressive activity of spleen T cells from C57BL/6 mice 14 days after the injection of 3 mg BCG

C57BL/6 spleen cells	Number of cells per well	cultivated			suppression (%)	
		alone	+ irradiated DBA/2	+ non-irradiated DBA/2	irradiated DBA/2	non-irradiated DBA/2
Normal T	$5.0x10^5$	6,310 ± 1,166	87,681 ± 11,313	99,893 ± 17,085		
BCG T	$5.0x10^5$	546 ± 915	28,446 ± 1,019	59,669 ± 8,308		
Normal spleen cells	$5.0x10^5$	9,042 ± 1,256	48,982 ± 1,951	55,895 ± 3,168		
Normal spleen cells + BCG T	$5.0x10^5$ + $5.0x10^5$	4,794 ± 1,045	41,825 ± 1,756	48,972 ± 8,034	54	52

Table 3. Suppressive activity of adherent spleen cells from C57BL/6 mice 14 days after the injection of 3 mg BCG

C57BL/6 spleen cells	Number of cells per well	cultivated			suppression (%)	
		alone	+ irradiated DBA/2	+ non-irradiated DBA/2	irradiated DBA/2	non-irradiated DBA/2
Normal adherent	$2.5x10^5$	143 ± 13	1,406 ± 102	27,532 ± 3,455		
BCG adherent	$2.5x10^5$	202 ± 21	192 ± 36	604 ± 90		
Normal spleen	$2.5x10^5$	181 ± 38.5	10,828 ± 1,445	30,294 ± 8,329		
	$5.0x10^5$	924 ± 28.9	13,483 ± 4,323	14,995 ± 266		
Normal spleen + normal adherent	$2.5x10^5$ $2.5x10^5$	856 ± 159	8,922 ± 1,508	16,123 ± 1,018		
Normal spleen + BCG adherent	$2.5x10^5$ $2.5x10^5$	351 ± 62	359 ± 108	375 ± 196	100	100

In this experiment the medium was not supplemented with 2-mercaptoethanol

We attempted to define some physiological characteristics of the cells which possess such suppressive properties. They were thus either irradiated (900 rad) or heated (56°, 30 min). It can be seen that after both treatments of adherent cells, considerable suppressive activity was conserved (Tables 4 and 5).

- Effect of a previous injection of BCG on the spleen cell responsiveness in graft-versus-host reactions.

C57BL/6 mice received an intravenous injection of 3 mg BCG 7 or 14 days before the test. Untreated mice were included as controls. The spleens were harvested and dissociated ; the spleen suspensions were pooled for each donor group and equal number of cells ($5x10^6$ or 10^7) were injected into irradiated (C57BL/6 x DBA/2)F1 hybrids. Table 6 shows the graft-versus-host reaction at its peak on day 4 as measured by the amount of ^{125}IUDR uptake in the lymph nodes and the spleen of the recipient.

The GVH induced by spleen cells from BCG treated mice harvested 14 days after injection was of a much lower intensity than that induced by control cells.

In an attempt to define the type of cell responsible for our observation we purified the spleen cell suspension and repeated the experiment with spleen T cells. It can be seen in Table 6 that the splenic ^{125}IUDR incorporation was much smaller when the GVH was induced by T cells from mice injected 14 days earlier with 3 mg BCG.

Since such a depressed reactivity could be due either to a lack of responsiveness of the treated cells, or to their suppressive properties, experiments were performed to determine whether cells from BCG-treated mice could depress the reactivity of normal cells. When BCG-treated spleen cells were added to normal spleen cells or when BCG-treated macrophages were added to normal T cells, the percentage of ^{125}IUDR incorporated into the spleen of the recipients was not significantly lower than the sum of the values obtained when the cells were injected separately, thus suggesting a lack of active suppression in vivo.

Discussion :

The suppression induced by the administration of high doses of BCG to mice non-specifically affects several types of immune activities. We have previously reported a decrease in the PHA responsiveness and in a comparable situation, Bullock, Carlson & Gershon showed that spleen cells from C3H/Anf mice infected by Mycobacterium lepraemurium suppressed the direct plaque-forming cell response of normal spleen cell cultures to sheep erythrocytes.

Table 4. Influence of irradiation on the suppressive activity of adherent spleen cells from C57BL/6 mice 14 days after the injection of 3 mg BCG

C57BL/6 spleen cells	Number of cells per well	cultivated			suppression (%)	
		alone	+ irradiated DBA/2	+ non-irradiated DBA/2	irradiated DBA/2	non-irradiated DBA/2
Irradiated normal adherent	$2.5x10^5$	15 ± 9.5		20,081 ± 1,834		
Irradiated BCG adherent	$2.5x10^5$	26.6 ± 8 1		655 ± 135		
Normal spleen + irradiated normal adherent	$2.5x10^5$ $2.5x10^5$	15 ± 1	17,388 ± 902	24,377 ± 2,343		
Normal spleen + irradiated BCG adherent	$2.5x10^5$	17.4 ± 7.5	163 ± 117	970 ± 602	99	96

The medium was not supplemented wth 2-mercaptoethanol.

Table 5. Influence of heating on the suppressive activity of adherent spleen cells from C57BL/6 mice 14 days after the injection of 3 mg BCG

C57BL/6 spleen cells	Number of cells per well	cultivated			suppression (%)	
		alone	+ irradiated DBA/2	+ non-irradiated DBA/2	irradiated DBA/2	non-irradiated DBA/2
Heated normal adherent	$2.5x10^5$	20.3 ± 0.6	116 ± 13.6	14,210 ± 2,018		
Heated BCG adherent	$2.5x10^5$	22 ± 1 7	129.6 ± 40.4	6,458 ± 399		
Normas spleen + heated normal adherent	$2.5x10^5$ $2.5x10^5$	17 5 ± 4 3	15,847 ± 807	25,794 ± 1,978		
Normal spleen + heated BCG adherent	$2\ 5x10^5$ $2.5x10^5$	29.7 ± 5 4	4,666 ± 838	7,849 ± 470	71	70

The medium was not supplemented with 2-mercaptoethanol

Table 6. ^{125}IUDR incorporation in the lymph nodes and spleen cells of lethally irradiated (C57BL/6xDBA/2)F1 mice 4 days after the injection of spleen cells or of spleen T cells from C57BL/6 mice

Treatment	Injected cells	^{125}IUDR incorporation x 10^3			
		Lymph nodes	P	spleen	P
None	$5x10^6$ spleen	7.11 ± 5.0		122.3 ± 38.0	
			⩽ 0.025		⩽ 0.001
3mg day 14	$5x10^6$ spleen	2.3 ± 1.2		22.2 ± 15.5	
None	10^7 spleen	14.3 ± 6.3		139.9 ± 12.8	
			⩽ 0.02		⩽ 0.001
3 mg day 14	10^7 spleen	6.2 ± 4.2		63.1 ± 18.4	
None	$5x10^6$ spleen T	12.1 ± 4.0	-	142.9 ± 17.8	-
3 mg day 7	$5x10^6$ spleen T	5.8 ± 2.4	⩽ 0.02	140.4 ± 15.0	NS
3 mg day 14	$5x10^6$ spleen T	1.5 ± 0.2	⩽ 0.001	15.5 ± 15.0	⩽ 0.001

Watson, Slivic & Brown (1975) have suggested that the poor antibody response to SRBC observed in vivo and in vitro during M. lepraemurium infection is caused by defective macrophage function at the level of antigen processing due to overloading of the macrophage with mycobacteria (Rojas-Espinosa, Casoluengo-Mendes & Villaneuva-Diaz, 1976). This is not likely to be the case in our studies, since we observed that not only are spleen cells unable to multiply but that they are also extraordinarily suppressive, the highest efficiency being observed among the adherent population. A less intense activity is observed in the nylon-purified T cells, in conditions where a contamination by adherent cells seems rather unlikely, but is impossible to exclude. The suppressive activity of BCG-treated C57BL/6 cells is non-specific, as both one- and two-way MLC reactions were inhibited. The heat resistance of the suppression caused by the adherent cells might be compared to the observation reported by Ptak & Gershon (1975) who noted that in Mishell-Dutton cultures the addition of heat-killed macrophages is suppressive.

In vitro, the ^{125}IUdR incorporation in GVH reactions after the injection of BCG spleen cells was lower than after the injection of non-treated cells. This was also the case when nylon-purified T cells were used. This cannot be due to a dilution of the reactive cells in the splenomegaly observed 14 days after BCG injection, as a proliferation of all cell types occurs and the proportional yield of T cells after nylon purification is comparable to normal.

We would like to stress the differences between the in vitro and in vivo results, as far as active suppression is concerned. While it was very easy to show significant suppression in vitro by adding BCG-treated cells to normal in a mixed-lymphocyte culture, we have not been able to demonstrate this in vivo.

The reason for this failure is perhaps technical, and may be due, for example, to a lack of suitable contact between the suppressive and responding cells.

Our experimental protocol in which spleen cells are successively stimulated by two antigens, BCG and histocompatibility antigens, is comparable to those intended to elicit the development of an antigenic competition in which the injection of a first antigen leads to an important decrease in the response to a second unrelated antigen.

The T-cell dependency of such an observation has been discussed (Gershon & Kondo, 1971 ; McArthur, Siskind & Thorbecke, 1974 ; Monier, 1975),

and it also seems fair to assume that T-cell activated macrophages could be held responsible (Sjöberg, 1972).

Our present results could very well be reported as an example of antigenic competition in which active suppression may be clearly demonstrated in vitro, and is essentially present in the adherent population, while no active suppression was seen when the treated cells were transferred in vivo.

PUBLICATION EDITEE DANS LE CADRE DU CONTRAT

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Immunology, 1980, 39, 263.

Titre du Projet N° 7 : Conséquences des irradiation à faibles doses.

Chef du Projet et Collaborateurs Scientifiques :
Claude JASMIN, A. MACIEIRA-COELHO, M. ALLOUCHE, C. DIATLOFF

BONE TUMOURS INDUCED IN RATS WITH RADIOACTIVE CERIUM

We have explored the possibility of using a radioinduced rat bone sarcoma as an experimental model : a very efficient technique has been developed by injecting ^{144}Ce close to the bone of the knee joint.

Our objectives were : (1) to trace the natural development of these bone tumours and their metastatic spread, especially to the lungs, (2) to examine the relationship between the time of appearance of the metastases and that of the primary tumour, as the occurrence of metastases in human osteosarcoma patients signifies a bad prognosis , and (3) to evaluate the cell proliferation kinetics of the primary tumours. These data, together with the available data on human bone sarcomas, may be useful in the developing better approaches to the therapy of these tumours.

Lung metastases were observed in 80% to 85% of rats bearing advanced malignant bone tumours (osteogenic osteosarcomas and angiosarcomas). These tumours were induced in 2-month-old Sprague-Dawley rats by inoculation of a colloidal suspension of radioactive cerium (^{144}Ce) into the hind leg, in close contact to the bones of the knee joint. Twenty eight rats were killed or died spontaneously shortly after detection of palpable tumours at the site of injection : the incidence of lung metastases was 73.3% and 53.8%, respectively, for osteogenic sarcomas and angiosarcomas, showing that most lung metastases are present at the time of diagnosis of the primary tumour. Tumour-cell kinetic parameters were studied in 49 rats bearing tumour following intraperitoneal injection of $\angle^{3}H\_\rangle$ thymidine. The labelling index (LI) of the primary tumours was significantly lower in advanced tumours (7.2% for ostosarcomas and 10.1% for angiosarcomas) than in tumours examined at the time of detection (12.2% and 13.5%, respectively). Mitotic indices (MI) of all tumours were less than 1%. From the curve of the percentage of labelled mitoses (PLM) at different times after $\angle^{3}H\_\rangle$ thymidine injection, T_S (6.5 h) and T_{G2} (1.75 h) were determined T_C and T_{G1} were also evaluated (18 h and 9.25 h, respectively). These results show that malignant bone tumours induced

in rats with ^{144}Ce may be a good model for human osteosarcomas and may be useful in studying the numerous problems in the therapy of malignant bone tumours in man.

Effect of low dose rate irradiation on the division potential of chick embryonic fibroblasts

Ionising radiation accelerates a natural phenomenon in cells with a limited growth potential (chicken) it shortens the lifespan and in cells that can acquire an unlimited growth potential (mouse) it accelerates the acquisition of the latter ; human fibroblasts showed an intermediate response since ionising radiation neither established the cultures as with mouse cells nor reduced the number of cells produced, as with chicken fibroblasts. Human cells also show an intermediate behaviour to chicken and mouse fibroblasts in the sense that chicken cells never establish either spontaneously or after treatment with virus, chemicals or radiations, mouse cells establish spontaneously, and human cells never establish spontaneously but can establish after infection with certain viruses. So they seem more prone to become established than chicken cells, but less than mouse cells.

It is difficult to see what could cause the different responses of the fibroblasts from these three species. It seems, however, that there are three possible explanations. It is possible that in the case of mouse fibroblasts there are some cells in the original population which have the potential of dividing indefinitely. If these cells are less radiosensitive, ionising radiation could favour their overgrowth by killing or retarding the growth of the other cells in the population which have a limited doubling potential. In that case, one would have to infer that in chicken fibroblast populations there are not cells with an infinite doubling potential. On the other hand, among human fibroblasts there would be clones with different growth potentials, and ionising radiation would favour the clones with the longest division potential.

The other hypotheses would concern the presence of absence of repair mechanisms or of a reparable substrate. It has been suggested that malignancy could be due in certain cases to mutations caused by faulty repair and that situations inducing repair therefore increase malignant transformation. Repair induced by low dose rate ionising radiation would cause mutations which determine the capacity to divide indefinitely. The situation would be analogous to what happens with Micrococcus radiodurans that becomes resistant to ionising radiation, because it develops the capacity to repair quickly the DNA lesions. If these were the case one would have to postulate that cells like mouse fibroblasts are endowed with the capacity to spontaneously undergo these changes, which would be accelerated by ionising radiation. Cells like chicken fibroblasts on the other hand, are endowed with a finite lifespan, perhaps because they have no repair enzymes or because the substrate (for example, DNA) has a molecular organisation which would impede the action of these enzymes. In any case the results seem to provide further evidence that a finite or infinite lifespan is an intrinsic property of cells and is not just dependent on a particular environment.

With the same methodology, we attempted to determine whether fibroblasts from human donors at high risk of cancer would also respond differently to radiation.

Skin fibroblasts from normal donors, donors with ataxia-telangiectasia or Fanconi's anemia, and from 1 cancer patient were treated with repeated γ-radiation at about 16 rads per hour. The remaining division potential of all fibroblasts, except for the Fanconi's anemia cells, was reduced to different extents by radiation. The growth potential of Fanconi's anemia cells was increased in all the irradiated culture. The increase was 54% in the group that survived the longest. These results were identical to those obtained with fibroblasts from certain species that have a high probability of transformation.

The results obtained with the Fanconi anemia fibroblasts are interesting because it is the first time that the growth potential of human fibroblasts is increased to such an extent by ionizing radiation. As it has been suggested that 1% of all persons dying from cancer and 5% of all patients dying from acute leukemia could be Fanconi anemia heterozygotes, the methodology described herein could also eventually be used to secreen a fraction of the population prone to cancer.

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Titre du projet n°8 : Experimental and clinical screening for agents capable of inducing immunorestoration.

Chef du projet et collaborateurs scientifiques : Georges MATHE, Irène FLORENTIN, Martine BRULEY-ROSSET, Antoine GOUTNER

The appropriate model for screening for agents capable of inducing immunorestoration is difficult to define, because it should use immunodepressed animals. Among the various possibilities (immunodepression induced by drugs, by sublethal irradiation, by chronic graft-versus-host reaction, in tumor-bearing animals) we selected the ageing process as being able to induce a long lasting physiological depression of the immune response.

The first part of the study tested the effects of two chemically defined compounds, azimexon and tuftsin on the response of immunocompetent mice. The second part tested the restoration of impaired immune functions of aged animals by chronic levamisole and bestatin administration.

The third part was a phase I study of azimexon in immunodepressed cancer patients.

In vivo immunomodulating properties of two synthetic agents : azimexon and tuftsin in immunocompetent mice.

Azimexon, an aziridine derivative, has already been demonstrated to possess some immunomodulating properties. In vivo, it potentiated delayed-type hypersensitivity reaction and humoral response to sheep red blood cells. It reduced the immunosuppression induced by cyclophosphamide or irradiation, and it displayed antitumor activity. In vitro, modulation of lymphocyte response to T-cell mitogens and macrophage activation were observed after incubation of the cells with this agent.

Tuftsin is a basic tetrapeptide which is responsible for the phagocytosis-stimulating activity of a leukophilic IgG to which it is covalently bound. Congenital or acquired deficiency in metabolism of this peptide coincides with a high incidence of infection. Its chemical structure is L-threonyl-L-lysyl-L-prolyl-L-arginine, and different methods of synthesis have been described. The immunomodulating properties of tuftsin are suggested by the observation that it increased resistance to bacterial infections and to tumor growth when injected into mice. In vitro, it modified the behavior

of macrophages and of polymorphonuclear cells. This study attempts to further elucidate the mode of action of azimexon and tuftsin.

Mice were submitted to various immunologic tests at different times after a single intravenous (i.v.) injection of azimexon or tuftsin in order to determine the mode of action of these chemically defined immunomodulators.

Azimexon, (BM 12 531) potentiated antibody responses to both thymus-dependent (TNP-KLH) and thymus-independent (TNP-LPS) antigens and DTH reaction to oxazolone when injected at least 1 day before the antigen. It activated macrophages, rendering them cytostatic for tumor cells, but depressed ADCC activity of spleen cells directed against antibody-coated CRBC.

Tuftsin, a basic tetrapeptide, potentiated antibody response to TNP-KLH when injected at least 3 days before the antigen. The response to TNP-LPS was stimulated on days 1 and 3, but was slightly depressed on day 7. It rendered macrophages highly cytostatic for tumor cells but, as observed with azimexon, the activation process required 7 days to develop. ADCC was enhanced throughout the period of observation.

Nonspecific suppressor cells were not detectable in the spleen of azimexon- or tuftsin-treated animals.

Table 1. Effect of azimexon and tuftsin on the antibody responses to a thymus-dependent antigen (TNP-KLH) and to a thymus-independent antigen (TNP-LPS).

Agent	Antigen	Day of administration before the antigen				
		0	1	3	7	10
Azimexon	TNP-KLH	1.0[a]	1.9*[b]	1.5*	1.7*	2.3*
	TNP-LPS	0.8	2.2*	1.3*	2.1*	1.7*
Tuftsin	TNP-KLH	0.9	1.0	2.4*	3.1*	1.5*
	TNP-LPS	1.2	1.8	1.4*	0.8*	1.0

[a] $\frac{\text{Mean number of anti-TNP-PFC/spleen in agent-treated mice}}{\text{Mean number of anti-TNP-PFC/spleen in control mice}}$

b* The response of the agent-treated mice is significantly different from that of control mice.

Table 2. Effect of azimexon and tuftsin on the spleen cell response to mitogens

Agent	Mitogen	Relative response of agent-treated spleen cells Day of administration of the agent			
		1	3	7	10
Azimexon	-	2.26*[a,b]	2.01*	2.06*	1.92
	PHA	0.79*	0.80*	0.80*	0.70*
	LPS	0.47*	0.47*	0.37*	0.86*
Tuftsin	-	1.46*	1.74*	1.83*	1.39*
	PHA	1.27*	0.82*	0.59*	0.49*
	LPS	0.80*	0.56*	0.75*	0.77*

[a] $\frac{\text{Mean cpm in cultures of } 5 \times 10^5 \text{ agent-treated spleen cells}}{\text{Mean cpm in cultures of } 5 \times 10^5 \text{ normal spleen cells}}$

[b] * Significantly different from control responses.

Table 3. Effect of agent-treated spleen cells on the mitogen responsiveness of normal spleen cells

Agent	Mitogen	Relative response of normal spleen cells cocultivated with agent-treated spleen cells Day of administration of the agent			
		1	3	7	10
Azimexon	-	1.00[a]	1.16	0.92	1.56*[b]
	PHA	1.02	1.04	0.94	1.02
	LPS	1.15	1.07	0.87	0.97
Tuftsin	-	0.87	0.86	1.17	1.25*
	PHA	1.25	1.04	0.90	0.96
	LPS	1.09	1.07	0.82	0.80

[a] $\frac{\text{Mean cpm in cultures of } 5\text{x}10^5 \text{ normal spleen cells admixed with } 2.5\text{x}10^5 \text{ agent-treated spleen cells}}{\text{Mean cpm in cultures of } 7.5\text{x}10^5 \text{ normal spleen cells}}$

[b] * Significantly different from control responses.

Table 4. Effect of azimexon and tuftsin on macrophage cytostatic activity on L1210 leukemic cells

Agent	Day of administration of the agent		
	3	7	10
Azimexon	0%[a]	50%	65%
Tuftsin	0%	95%	94%

[a] Cytostatic index = 100 - $\frac{^3\text{H-TdR incorporation by tumor cells exposed to agent-treated macrophages}}{^3\text{H-TdR incorporation by tumor cells exposed to normal macrophages}} \times 100$

Table 5. Effect of azimexon and tuftsin on the antibody-dependent cellular cytotoxicity against antibody-coated chick erythrocytes

Agent	Day of administration	LU_{50}[a] value ($\times 10^4$)	Number of LU/spleen
Azimexon	1	23	220
	3	30.5	240
	14	32	210
Controls	-	12.5	460
Tuftsin	1	21	417
	3	32	325
	7	26	411
	14	34	314
Controls	-	41	195

[a] Number of spleen cells required to lyse 50% of 10^4 target cells

- Restoration of impaired immune functions of aged animals by chronic levamisole and bestatin treatment.

Alteration of the immune system in the ageint process is thought to be responsible either directly or indirectly for some diseases of ageing such as increase of autoimmune manifestations, incidence of infections and neoplasias. This alteration affects mainly the T-cell compartment and that it could be related to thymus involution which occurs early in life. B-cell responses were affected to a lesser degree whereas macrophage functions remained unchanged. Delay, reversal or prevention of the decline in normal immune functions may retard the onset or lessen the severity of the diseases of ageing.

The following experiments deal with an assay for restoring the immune capacity of aged immunodepressed mice by a chronic treatment with bestatin and levamisole. Aged mice were found to have depressed T-cell and B-cell responses but increased ADCC activity. Weekly injections of bestatin over a period of 6 months in (C57BL/6 x BALB/c)F1 mice resulted in varying effects depending on the dose administered. Small doses (10 µg per injection) were more effective in restoring humoral responses to SRBC rather than delayed-type hypersensitivity reactions, whereas large doses (100 µg per injection) acted in the opposite way. Macrophage activation was only obtained after the administration of the high doses of bestatin. Continuous treatment with bestatin did not prevent the appearance of suppressor cells induced by ageing. It led to a significant reduction of ADCC activity in aged animals near to the base line value of young animals. Animals were examined for the presence of spontaneous tumors from the end of the treatment until the age of 28 months. A significant reduction of spontaneous tumor incidence was observed in mice given repeated injections of 100 µg bestatin when compared to untreated aged mice and to mice given the low doses of bestatin (Fig. 1B).

Continuous treatment of (C57BL/10 x DBA/2)F1 mice with levamisole restored T-cell dependent functions (delayed-type hypersensitivity reaction and antibody response to T-dependent antigens) and led to macrophage activation. This treatment significantly reduced ADCC activity near the baseline value of young mice. In addition, levamisole prevented the appearance of suppressor cells induced by aging. As did bestatin treatment, levamisole increased the life span of the animals and reduced the spontaneous occurrence of tumors (Fig. 1A).

Table 1. Effect of bestatin administration to aged mice on macrophage activation. In vitro cytostatic activity of peritoneal macrophages for lymphoid leukemic cells.

		Dose of bestatin per injection*	
	untreated mice	10 μg	100 μg
Mean cpm ± SE	19,687 ± 4,898	18,937 ± 4,048	3,416 ± 1,396
Inhibition of tumor cell proliferation (%)	-	4	83
Significance		$P > 0.05$	$P < 0.001$

* Mice were given 10 or 100 μg of bestatin weekly from the age of 16 months, over a period of 6 months.

$$100 \times \frac{\text{cpm in cultures of untreated macrophages} - \text{cpm in cultures of bestatin-treated macrophages}}{\text{cpm in cultures of untreated macrophages.}}$$

Table 2. Effect of bestatin administration to aged mice on the plaque forming cell (PFC) response to sheep red blood cells

		Dose of bestatin per injection*	
	untreated mice	10 μg	100 μg
Mean number of PFC/ spleen ± SE[a]	26,800 ± 17,200	64,200 ± 22,600	36,000 ± 28,600
Significance		$0.02 < P < 0.01$	$P > 0.05$

* Mice were given 10 or 100 μg of bestatin weekly from the age of 16 months over a period of six months.

[a] Ten animals per group.

Table 3. Effect of bestatin administration to aged mice on delayed-type hypersensitivity to oxazolone

	Untreated 2-month-old mice	Untreated 22-month-old mice *	Dose of bestatin per injection*	
			10 µg	100 µg
Mean ear thickness increment ± SE[a] (1/100e mm)	7.67 ± 1.76	3.71 ± 0.95	1.75 ± 0.38	9.38 ± 1.84
Significance		$P < 0.01$	$P > 0.05$	$0.05 < P < 0.02$

* Mice were given 10 or 100 µg of bestatin weekly from the age of 16 months over a period of six months.

[a] Five animals per group.

Table 4. Effect of bestatin administration to aged mice on antibody-dependent cellular cytotoxicity against ^{51}Cr-labelled CRBC

Mice	Treatment	Percentage specific lysis (± SE) for different effector:target cell ratio			LU_{60}[a] per culture ($\times 10^{-4}$)	Number of LU_{6} per spleen
		100:1	50:1	25:1		
Young mice	-	66±1.7	66±1.7	49±1.7	38	184
Aged mice	-	86±0.5*	78±2.9*	66±1.7*	21	561
Aged mice	10 µg bestatin	70±2.3*	71±2.3*	58±1.7*	29	324
Aged mice	100 µg bestatin	65±1.7	57±1.1	55±1.7*	65	218

* Significantly higher than the values observed in the group of young mice ($P < 0.001$).

[a] Number of spleen cells required to lyse 60% of 10^4 ^{51}Cr-labelled CRBC

Table 5. Delayed hypersensitivity reaction to oxazolone of 18-month-old mice treated with levamisole

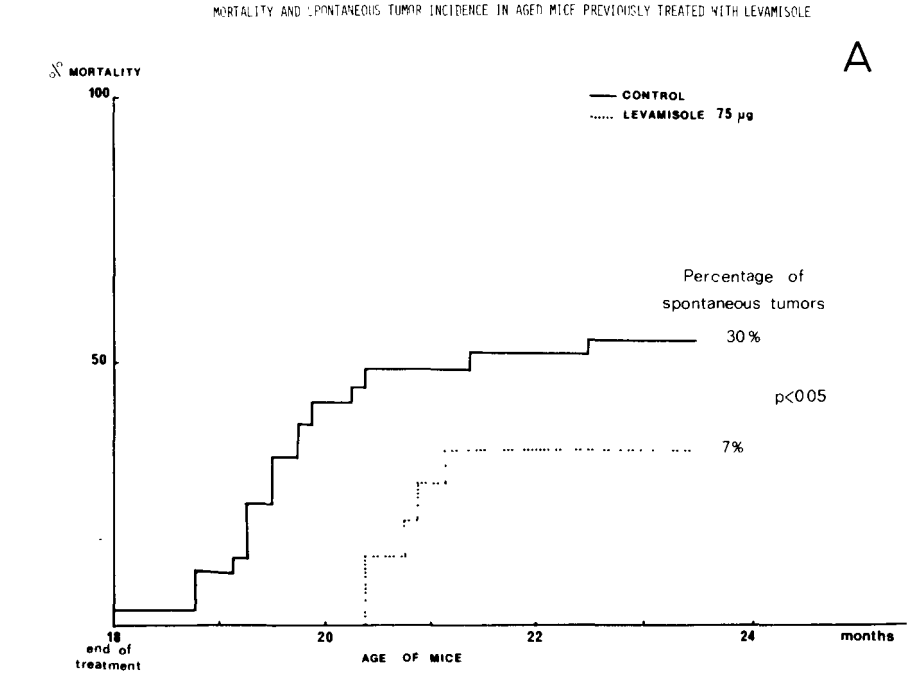
Treatment	Ear thickness increment (1/100 mm) ± SE	
Untreated young mice	7.67 ± 1.76	
Untreated aged mice	2.13 ± 0.24	
Levamisole-treated aged mice	6.60 ± 1.04	$P < 0.01$

Table 6. Macrophage activation measured by cytostatic activity for tumor cells and antibody response to SRBC in 18-month-old mice treated by levamisole

	18-month-old mice	
	Untreated	Levamisole-treated
Macrophage activation		
Mean cpm ± SE	35,339 ± 7,845	17,430 ± 4,754
% inhibition of tumor cell proliferation	-	51%
Antibody response		
Mean number of PFC/spleen to SRBC ± SE	18,333 ± 3,400	47,108 ± 12,945

Table 7. Effect of levamisole administration to aged mice on antibody-dependent cellular cytotoxicity

Treatment	% specific lysis Effector:target cell ratio			
	100:1	50:1	25:1	12:1
Untreated young mice	58 ± 4	55 ± 1	45 ± 5	20 ± 2
Untreated aged mice	70:1	72 ± 2	60 ± 4	45 ± 3
Levamisole-treated aged mice	59 ± 2	53 ± 4	47 ± 4	43 ± 4


MORTALITY AND SPONTANEOUS TUMOR INCIDENCE IN AGED MICE PREVIOUSLY TREATED WITH LEVAMISOLE
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50
CONTROL
LEVAMISOLE 75 µg
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spontaneous tumors
30 %
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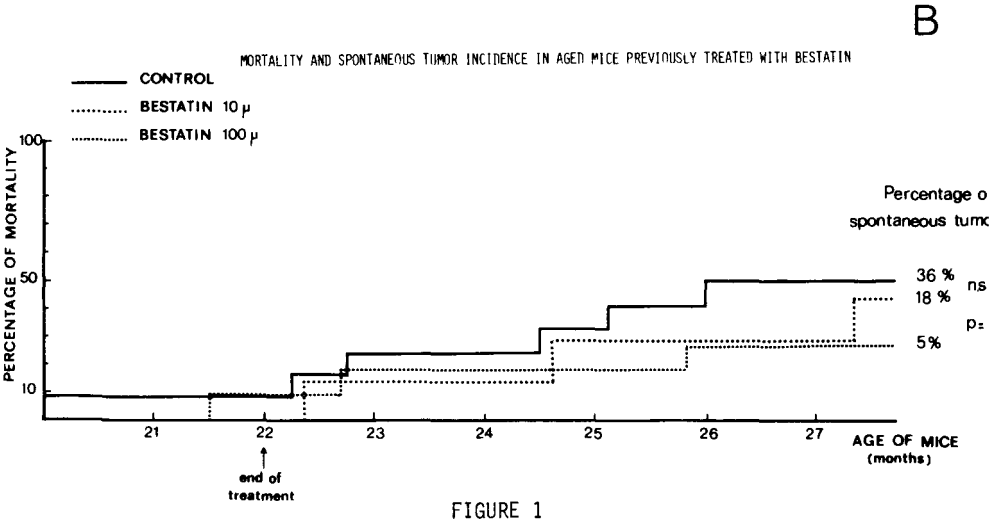
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MORTALITY AND SPONTANEOUS TUMOR INCIDENCE IN AGED MICE PREVIOUSLY TREATED WITH BESTATIN
CONTROL
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treatment
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FIGURE 1

- Phase I study of azimexon in immunodepressed cancer patients.

Sixteen patients bearing various solid tumors and seven breast cancer patients received azimexon. Azimexon administration was very well tolerated and no patient complained of nausea or vomiting. No sign of renal, cardiac, hepatic, neurologic toxicities were observed. No modification of the biological parameters could be attributed to the administration of Azimexon.

Among 16 patients bearing solid tumors, fifteen of them were totally anergic, one of them was hypoergic, 10 of the anergic patients experienced a positivation of their DTH reactions. The hypoergic patient exhibited a reactivity well in the normal range after azimexon administration. Thus eleven out of 16 patients bearing solid tumors were immunorestored as far as their DTH reaction was concerned.

Among the seven immunodepressed breast cancer patients in the imperceptible phase, six were anergic and one was hypoergic. After azimexon administration 5 out of the 6 anergic patients displayed positive DTH reactions and the reaction of the hypoergic patient returned to the normal range.

Mitogen responsiveness was studied in 12 of the patients bearing advanced solid tumors. The results obtained in the 5 patients who remained anergic and in the 7 patients who were restored after azimexon administration indicate that, before azimexon treatment, the TPA responsiveness is depressed in 8 out of 12 cases while the PHA responsiveness is abnormal in only four cases.

After azimexon administration in the 5 patients remaining anergic, the proliferative response to TPA was either abolished, or deeply depressed as in the patient who had a normal reactivity to TPA before azimexon administration. In contrast, all the seven restored patients had normal TPA responses after azimexon administration. In four of them the level of reactivity was clearly abnormal before azimexon administration. In one case the PHA responsiveness was not simultaneously restored.

The spontaneous cell mediated cytotoxicity studied in 8 patients (6 patients bearing solid tumors, 2 breast cancer patients in two cases continued to decline : these two patients did not exhibit a restoration of their DTH reaction. In the six remaining cases the NK activity rose after

azimexon administration. The NK activity of patients 5 and 6, which was normal at the start, reached a high level after azimexon application, comparable to the stimulation that we observed in interferon-treated patients.

The results show that azimexon at the dose employed, is non toxic and has powerful immunorestorating properties. The delayed type hypersensitivity reactions were restored in 11 out of 16 patients bearing solid tumors and in 6 out of 7 breast cancer patients in the imperceptible phase. Although DTH reactions are probably not directly involved in the defense mechanisms against tumor cells, anergy in cancer patients is a factor of bad prognosis. The results of chemotherapy can be influenced by the DTH status of the patients. Thus this action on the DTH value might be of therapeutic importance.

Azimexon is now studied in immunochemotherapy randomized trials.

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Contractor: Istituto di Ricerche Farmacologiche "Mario Negri", Milan, Italy

Contract No.: CEE 221-761 BIOI

Research Groups Head: prof. Silvio Garattini

Theme of contract: CONSEQUENCES OF RADIATION EXPOSURE: PREVENTION AND TREATMENT OF PATHOLOGICAL EFFECTS

Project No.1: Characterization of the immunological effect of cytotoxics

Project leader: F. Spreafico, M.D.; A. Tagliabue, M.L. Moras, G. Conti

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Impairment of the activity of the immune system is a major consequence of exposure to radiation and radiomimetic compounds. Accordingly, a main line of investigation in these years has been directed to obtain a more in depth characterization of the effects of radiomimetic cytotoxics on the various subpopulations of the immune system. In addition to the aim of obtaining a better understanding of the physiology of the immune apparatus, a further rationale for these studies was the obtainement of a better insight into the mode of action of radiomimetics which could also be of interest in towards a better therapeutic use of such compounds. It is well known in fact that immunodepressive agents can be of therapeutic interest in conditions involving bone marrow transplantation.

On this general background, one line of investigation was directed to examine the effect of a number of cytotoxic agents, chosen as representative of the major classes of immunodepressants, on Natural Killer (NK) cells. Results obtained indicate that a significant heterogeneity exists among cytotoxic drugs in their activity on this type of immune defence mechanism. For instance, whereas the anthracycline Daunomycin in vivo was significantly inhibitory of NK activity in the mouse, its close structural analog Adriamycin was not inhibitory. Inhibition was still greater with Azathioprine and Cyclophosphamide, whereas the administration of DTIC was not associated with reductions in NK activity even at doses possessing potent and long-lasting depressive activity on the function of other immunocytes. A similar degree of heterogeneity among cytotoxic agents has also been documented by this group for another cell type involved in the resistance of the organism to different attacks, i.e. macrophages.

Adriamycin and Cyclophosphamide for instance were relatively sparing of these cells, as assessed in terms of their cytotoxicity towards cultured cells, whereas Daunomycin and DTIC were markedly inhibitory. Another cell type on which various cytotoxic immunodepressive agents were investigated with regard to their possible modulatory activity, has been T suppressor cells involved in the regulation of antibody formation (Ts-abs), demonstrating again marked variations among compounds, as for instance examplified by the insensitivity of these cells and their precursors to Daunomycin, in contrast to the potent activity of Adriamycin. Cyclophosphamide was clearly inhibitory, whereas clearly less active was Azathioprine. More recently, it has been demonstrated that specific T suppressor involved in the regulation of cell-mediated reactivity (Ts-dth) are not only also variably sensitive to inhibition to cytotoxics, but are in turn differently sensitive to certain (but not all) compounds in comparison to Ts-abs,even if evoked by the same antigen.

From these data it appears therefore that cytotoxic agents,even when structurally closely related and believed to have the same biochemical mode of action, are substantially heterogeneous in their interaction with different populations and subsets of immunocytes, a type of information which has potential significance in the development of less empirical uses of these agents, as well in understanding their biological activities.

In studies complementary to those described, the capacity of a novel immunodepressant with great therapeutic potential, Cyclosporin A, to influence cells involved in natural resistance mechanisms, was investigated using both rodent and human cells,showing that this compound is relatively sparing of these effector mechanisms. In the search for novel less toxic immunodepressants, two preparations of Batroxobin, snake venoms with defibrinating activity were studied. These agents showed remarkable immunodepressiv activity in vitro and in vivo on humoral as well as cell-mediated reactivities through a non cytotoxic but still undefined mechanism. As a follow-up of experimental studies on the resistance of macrophage functions to given cytotoxic agents, investigations were also conducted in humans therapeutically given the same compounds and obtaining evidence confirming results in rodents while also demonstrating the capacity of human cancer-associated macrophages to stimulate the growth of culture neoplastic cells. Lastly, the presence of NK activity in intestinal mucosa has been desctibed and the association of this activity with a distinct type of lymphoid cell characterized, as a first step in an analysis of the local immune defence systems at sites known for their sensitivity to radiation or radiomimetic damage.

List of papers produced in the period 1976-1980 under support of Euratom grant CEE 221-76-1 BIOI:

- A. Mantovani, N. Polentarutti, P. Gritti, G. Bolis, A. Maggioni, F. Spreafico: K cell activity in ovarian cancer patients given chemotherapy. Europ.J.Cancer 15: 797-802 (1979).
- A. Mantovani, W. Luini, G. Peri, A. Vecchi, F. Spreafico: The effect of chemotherapeutic agents on natural celle mediated cytotoxicity in mice. J.Natl.Cancer Inst. 61: 1255-1261 (1978)
- A. Vecchi, M. Sironi, F. Spreafico: Preliminary characterization in mice of the effect of Isoprinosine on the immune system. Cancer Treat.Rep. 62: 1975-1979 (1978)
- A. Mantovani, G. Peri, N. Polentarutti, G. Bolis, C. Mangioni, F. Spreafico: Effects on in vitro tumor growth of macrophages isolated from human ascitic ovarian tumors. Int.J.Cancer 23: 157-164 (1979)
- A. Anaclerio, G. Conti, G. Goggi, M.L. Honorati, A. Ruggeri, M.L. Moras, F. Spreafico: Effect of cytotoxic agents on suppressor cells in mice. Europ.J.Cancer 16: 53-58 (1980)
- A. Anaclerio, A. Ruggeri, A. Poggi, F. Spreafico, M.B. Donati: In vivo and in vitro immunosuppressive effect of two batroxobin preparations in mice. Thromb.Res. 18: 253-258 (1980).
- A. Gescher, M. D'Incalci, R. Fanelli, P. Farina: N-hydroxymethylpentamethylmelamine, a major in vitro metabolite of hexamethylmelamine. Life Sci. 26: 147-154 (1980)
- M. Introna, P. Allavena, F. Spreafico, A. Mantovani: Inhibition of human natural killer activity by Cyclosporin A. Transplantation, 1981, in press
- A. Tagliabue, W. Luini, D. Soldateschi, D. Boraschi: Natural killer activity of gut mucosal lymphoid cells in mice. Cellular Immunology, submitted.
- F. Spreafico, A. Vecchi, G. Conti, M. Sironi: On the heterogeneity of immunotherapeutic agents. Proc. of the: 1st Intern.Conf. on 'Immunopharmacology' - Brighton, July 1980 , in press.
- F. Spreafico, A. Mantovani: Immunomodulation by cancer chemotherapeutic agents and antineoplastic activity. Pathobiology Annual 1980, H.L. Ioachim, Ed., Raven Press, N.Y., in press.
- F. Spreafico, M.L. Moras, A. Vecchi, S. Filippeschi, M. Sironi, A. Tagliabue: The immunostimulatory activity of B.subtilis spores in experimental animals. Chemiother.Oncol., in press, 1981.

Project No. 2: Search and Characterization of Immunomodulatory Agents

Project Leader and coworkers: F. Spreafico, M.D.; A. Vecchi, M. Sironi, S. Filippeschi

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A second main line of studies pursued under this contract regarded the search of and characterization of the mode of action of novel immunostimulatory agents. The rationale at the basis of these studies lies, briefly, in the potential of this type of pharmacological agents for the treatment of the immune damage consequent to exposure to excessive radiation. It is also well known that currently available agents with such an activity are limited in number and not entirely satisfactory for therapeutic use for a number of reasons, including toxicity, difficulty in standardization, complexity of structure, etc.

On this background, a first type of studies was targeted at the obtainement of a better understanding of their mode of action, as a guide to their safer and more effective employ both expeirmentally and clinically. In examining the effect of these agents on T suppressor cells, it was found that whereas some compounds (e.g. BCG, C.parvum, Pyran copolymer) could induce a marked and longlasting depletion of these cells from central compartments (e.g. thymus) reflecting a movement into more peripheral sites, other compounds (e.g. Levamisole) were ineffective in this regard. Subsequently, K cells (i.e. elements mediating antibody-dependent cellular cytotoxicity, ADCC), were identified as a possible important target population for the activity of at least some of these agents. However, it was also revealed that a substantial heterogeneity exists among these compounds in this type of activity in both qualitative and quantitative terms. The variability in the mode of action of biological response modifiers can have consequences in their choice for therapeutic use, as for instance revealed studying the immunological effects resulting from the use of combinations of such agents. It was in fact observed that in terms of therapeutic activity, evaluated as neoplastic growth inhibition, only combinations of agents having modes of action complementary at the cellular level can be synergistic, whereas non complementary combinations are non synergistic and can produce even paradoxical effects. In a further paper produced under support of this contract, a review is presented of the problems encountered in characterizing the mechanisms of their activity, in searching for new such drugs, in interspecies extrapolation of results and monitoring the effects of immunomodulatory agents.

Concomitantly with these basic studies, efforts were pursued in characterizing novel compounds possessing immunostimulatory capacity. A first chemical examined in experimental conditions as to its cell targets and therapeutic potential, was Isoprenosine. It was revealed that this synthetic agent, which possesses a clinically demonstrable activity in a variety of infectious disorders, is a clear immunostimulator, influecing directly T cells function in a manner comparable, under a series of respects, to thymic hormones. Another synthetic for which an immunostimulatory activity was revealed was 3-(p-chlorophenyl)-2,3-dihydro-3-hydroxythiazolo (3,2a)-benzimidazole. For this compound it was characterized the ability to increase antibody production to T-dependent (but not T-independent) antigens, to increase T-dependent cell-mediated reactivities in vitro and in vivo, whereas no effects were observed in directly influencing macrophage numbers or function nor on K cells. More recently, two other compounds of biological origin /a high M.W. glucan (Pustulan) and the spores of strain ATCC 1999 B subtilis/ were also revealed to be potent immunostimulators, their administration to animals producing very significant increases in immune reactivities, both humoral and cell-mediated, in both normal and hyporesponsive hosts challenged with a variety of stimuli, macrophages being a major cell target for their immunopharmacological activity. It is of note that for all compounds mentioned, the therapeutic index appears to be very high and that for at least one compound, therapeutic effect was observable also via the oral route.

Vertragspartner der Kommission :

Land Baden Württemberg, vertreten durch die Universität Ulm für die Abteilung Klinische Physiologie und Arbeitsmedizin
(Leiter : Prof. Dr. med. T.M. Fliedner)

Vertragsnummer :

222-76-1 BIO-D

Leiter der Forschungsgruppen :

Prof. Dr. T.M. Fliedner, Leiter der Abteilung für Klinische Physiologie und Arbeitsmedizin der Universität Ulm

Allgemeines Thema des Vertrages :

Consequences of Radiation Exposure : Evaluation and Treatment of Pathological Effects

Allgemeine Darstellung der durchgeführten Arbeiten :

The goal of the 5-year-Contract between the Commission and the Research Group at the University of Ulm, directed by Professor Dr. T.M. Fliedner was twofold. In a first project area, it appeared of interest to improve existing and develop new concepts and methods for the evaluation of the consequences of radiation exposure. In particular, the goal was to examine in what way the changes of hemopoietic stem cells in the peripheral blood after radiation exposure might be used as a biological indicator for the extend of hemopoietic radiation damage after whole and partial body exposure delivered at various doses and dose rates from external and internal sources. In addition it was felt, that quantitative and/or qualitativ blood stem cell changes after radiation exposure might be useful in predicting the rest function of hemopoiesis as well as late effects, such as development of neoplastic and non-neoplastic lesions. In a second project area, the study of hemopoietic restauration after whole body irradiation as a function of the type of stem cells used appeared to be of interest as well as the conditions for obtaining an allogeneic engraftment in the allogeneic situation. There was evidence, that stem cells might be obtained not only from bone marrow but also from the peripheral blood (by means of continuous cell centrifugation) and from fetal liver tissue. Thus, it appeared of interest to examine the possibilities and limitations of using blood stem cells for treatment of radiation induced bone marrow failure, to study the con-

ditions of stem cell cryopreservation (establishment of stem cell banks) and to evaluate possibilities and limitations in dogs and man to separate from mononuclear cell suspensions those hemopoietic stem cells endowed with pluripotent potentialities. The latter approach was thought to contribute to the problem of avoidance of graft-versus-host-reaction. It was also the objective to see in animal experimental and clinical studies, how far one can remove the bacterial flora from the intestinal tract and whether this type of bacterial decontamination might lead to a decrease in the severity of gvhd and particularly why.

The contract work was successfully executed between 1976 and 1980. Many new findings were obtained and published in the scientific literature. The detailed discussion of the results obtained in the 4 projects will show, that the initially set goals were approached successfully although - as usual in basic research - new problems and questions arise as a consequence of the work and will require continued scientific efforts.

Ergebnisse des Projektes/Results of Project No. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter (1976-1980:

Prof. Dr. T.M. Fliedner associated by Drs. W. Calvo, F. Carbonell, H. Gerhartz, G. Grilli, M. Haen, E.B. Harriss, M. Körbling, H. Kreutzmann, W. Nothdurft, H.-J. Seidel, K.H. Steinbach, P. Szemere et al.

Titel des Projektes/Title of Project:

Evaluation of pathophysiological consequences of radiation exposure by means of cell system changes, especially of the blood stem cell pool.

Darstellung der Ergebnisse/Presentation of results:

There is a need in radiation protection research to improve existing and develop new approaches that would allow one to predict the biological consequences of radiation exposure in the dose range below 100 rad of whole body exposure. The demonstration of hemopoietic stem cell migration streams through the blood connecting the various sites of bone marrow cell production lead to the question, whether blood stem cell changes may be useful as "biological indicators" or radiation exposure of the hemopoietic tissue and whether they may be of predictive value for the development of neoplastic and non-neoplastic consequences.

It is for these reasons that this project addressed itself to two questions: 1. What are the quantitative and qualitative characteristics of the hemopoietic stem cells present in mammalian blood and in relation to the extravascular stem cell pools. 2. What are quantitative and qualitative changes of the blood stem cell pool after single or repeated radiation exposures from external and internal radiation sources?

ad 1: It was the first objective to establish the normal content of hemopoietic stem- and/or progenitor cells in the blood of mice and dogs as well as in men. The "agar colony technic" was established and improved to measure the blood content of granulocytic progenitor cells as "CFU-C". In our hands, the blood of normal mice contains some 20 to 30 CFU-C and about 21 CFU-S per ml blood. In dogs, we find between 30 and 200 CFU-C per ml blood. In human beings, the normal values are between 30 and 200 per ml blood. In dogs and in man the blood samples were collected at regular intervals for 14 and 6 weeks, respectively. It was found that each dog and each person has its and his own characteristic blood CFU-C level which is maintained within individual limits. In dogs as well as in man, there was some evidence for systematic blood CFU-C oscillations. This was inter-

preted to mean, that the blood CFU-C concentration is a parameter regulated by certain feed back mechanisms. More recently, the technics for demonstrating in blood erythropoietic progenitor cells as "burst forming units of erythropoiesis" (BFU-E) were established. The BFU-E content of mouse blood is about 30 per ml blood. In man, the BFU-E concentration was found to vary between 100 and 800 per ml, thus being 3 - 5 times as high as the CFU-C.

A number of studies were performed to obtain information on some physical properties of progenitor cells in the blood and their migration and regulation pattern. In dogs, the blood CFU-C population is a small cell size subpopulation of those CFU-C forming the bone marrow population. This became evident when it was found that the bone marrow CFU-C sediment at a rate of 3.5 to 8 mm/hr while those of the blood sediment at a rate of 3 to 6 mm/hr. The ^{3}H-thymidine suicidal fraction of bone marrow CFU-C is in the range of 20 - 36 %, while that of the blood CFU-C is between 16 - 30 %. Dextran sulfate at a dose of 15 mg/kg body weight results in a dramatic increase in the blood CFU-C concentration in the blood by a factor of 10 within 3 hours, showing a "mobile" bone marrow CFU-C population ready for release into the blood. Mononuclear cell oriented leukocytapheresis (CFC) were performed in dogs for between 4 and 24 hours and showed that one can remove from the blood 60 - 80 times as many CFU-C as are present in the total blood volume without exhausting the bone marrow CFU-C pool. After CFC termination, there was a most remarkable CFU-C overshoot within 3 - 10 days before the blood CFU-C concentration returned to normal levels within 15 - 20 days. - In human beings, studies were performed to characterize some properties of blood CFU-C. When mononuclear cells, separated from normal human peripheral blood by the Isopaque-Ficoll method, were cultured in diffusion chambers (DC) and tested for the presence of CFU-C by re-culture of harvested DC cells in the agar colony method, a total increase of up to 25-fold was observed in 8 out of 10 persons. There was an immediate increase in CFU-C after chamber implantation into pre-irradiated host mice, independent of the initial number of CFU-C present in the implanted cell suspension, and the growth patterns were reproducible for individual persons. Furthermore, differentiation of cells in the DC culture system occurred not only into the granulopoietic/macrophage series but also into lymphatic cell lines and, even if rarely, into megakaryocytes and erythropoietic cells. Thus the

DC culture technique appears well-suited to test the ability of blood stem cells to reproduce and to differentiate into the recognizable hemopoietic cell lines.

It was of interest to observe, that - as in the dog - the blood CFU-C population of human peripheral blood is in a dynamic equilibrium with extravascular CFU-C populations, such as in the marrow. A 4-hour leukapheresis in man results in the collection of some $8.7 \pm 4.3 \times 10^5$ CFU-C. However, the concentration of blood CFU-C does not diminish significantly. This was taken to mean that the CFU-C removed are rapidly restored by CFU-C migration into the circulation. In man, there is also a reactive overshoot in the number of blood CFU-C. This has practical consequences since a leukapheresis performed 2 or 3 days after the first one will yield a higher number of CFU-C.

From all these findings it was concluded that the blood CFU-C represent a "physiological" subpopulation of blood mononuclear leukocytes and that their concentration at a certain point of time is the result of a dynamic equilibrium between CFU-C immigration from all active bone marrow sites and emigration or transformation.

ad 2: On this basis, a number of studies were performed in mice, dogs and men to examine the radiation response pattern of hemopoietic progenitor cells in the blood to single, repeated or low dose rate exposures.

The most extensive studies were performed in dogs and resulted in a D_o value of 25 ± 1 rad for blood CFU-C (when the blood was irradiated in-vitro) in comparison to a D_o value for bone marrow CFU-C of 61 ± 2 rad. This indicates that the blood CFU-C population may well be the most sensitive cell population of the mammalian organism. After whole body exposure with 20 rad, 40 rad and 80 rad, there is a dose related decrease of blood CFU-C within 24 hours and a subsequent slow but steady recovery.

To determine the radiosensitivity of hemopoietic progenitor cells in mouse bone marrow, a suspension of femoral bone marrow cells from CBA mice was devided into equal portions which were then irradiated in vitro with doses of 250 kV X-rays varying from 0 to 600 rad. The number of surviving granu-

lopoietic cells (CFU-C) in each portion was measured by the agar colony technique. From the resulting cell survival curve a D_o value of 100 rad for mouse bone marrow CFU-C was established.

The response of mouse blood stem cells to internal irradiation from ingested tritiated water (HTO) was determined. Young adult C_3H mice were maintained on HTO in drinking water over a period of 6 months and at monthly intervals measurements were made of the mature hemopoietic cells in the blood, the concentration of pluripotent stem cells (CFU-S) and of granulopoietic progenitor cells (CFU-C) in the peripheral blood and in the bone marrow. At a dose level of 6 µCi/ml, giving a whole body dose of about 1 rad/day, pluripotent and committed hemopoietic stem cells in peripheral blood and bone marrow were reduced to about half the normal levels. No significant effect on the mature cell counts in the blood was observed demonstrating the greater sensitivity of blood stem cell concentration as a biological indicator of radiation. At the dose level of 1 µCi/ml HTO in drinking water, no definite effect on hemopoietic stem cells in blood and bone marrow or on the mature cell counts in the blood could be demonstrated within the 6-month period of observation.

Thus, the blood stem- and progenitor cell changes in mice and dogs allow one to conclude, that they relate quite well to the radiation dose administered and are sensitive at doses and dose rates that would not be expected to cause changes of other blood cell populations.

Therefore, pilot studies were performed in human beings receiving radiation exposure for diagnostic or for therapeutic reasons. One group received Cobalt-60 partial body exposure (treatment of cancer), another group Technetium-99 m and a third group Jodine-131. Technetium administered for scintigraphic examination of the skeleton did not result in a decrease of blood CFU-C within a number of days, the estimated dose to the bone marrow being in the range of some 0.4 to 0.5 rad. In contrast, the administration of Jodine-131 at dosesof between 6 mCi and 74 mCi (resulting in an estimated bone marrow dose of 17 to 42 rad) resulted in a blood CFU-C decrease within 30 days to levels of 1/3 to 1/2 of normal. After external therapeutic partial body exposure, there was a marked blood CFU-C reduction within one week after the first exposure.

Thus, there is good evidence, that the blood progenitor cell populations may well serve as a useful indicator of whole and partial body radiation exposure. However, much further work needs to be done in order to establish the pathophysiology of the changes seen and their value in predicting early and late radiation consequences.

Ergebnisse des Projektes / Results of Project No. 2

Leiter des Projektes und wissenschaftliche Mitarbeiter (1976 - 1980) :

Prof. Dr. T.M. Fliedner associated by Drs. Calvo, F. Carbonell, H. Gerhartz, S.F. Goldmann, G. Grilli, H.D. Flad, M. Haen, W. Nothdurft, H.P. Schnappauf, E.B. Harriss, M. Körbling, W.M. Ross, I. Steinbach, D. Vassileva

Titel des Projektes / Title of Project :

Preclinical studies on the use of blood stem cells in the treatment of radiation - induced bone marrow failure

Darstellung der Ergebnisse / Presentation of results :

During 1976 - 1980 the major elements for the concept of a "blood stem cell bank" for the treatment of a radiation-induced bone marrow aplasia were established in a preclinical canine model. The major questions to be asked were the following : How can one improve the yield of the collection of hemopoietic progenitor cells from the blood by means of leukapheresis? How can one improve the efficiency of the cryopreservation of blood derived progenitor cells for instance by freezing large fluid volumes? What are the relationships between the number of progenitor cells transfused and the pattern of hemopoietic short and long term recovery in the autologous situation? Can one avoid a graft-versus-host-reaction (gvhd) in the allogeneic situation by means of transfusion of purified progenitor cell suspensions? What are the radiation-induced or stem cell transfusion-associated late effects to be observed in the marrow and in other tissues? For all questions, interesting and promising answers could be obtained which will serve as the basis for continuing studies.

The first question, related to methods to improve the yield of hemopoietic progenitor cells to be collected from the peripheral blood of dogs was answered in several ways. The injection of 15 mg/kg body weight of dextran sulfate (DS) resulted in a remarkable increase in blood CFU-C concentration within 3 hours by a factor of about 10. The number of CFU-C that can be collected by means of a 4-hour-leukapheresis (CFC) after dextran-sulfate administration can be increased by a factor of 60 as compared to leukapheresis without DS administration. Another approach is, to perform sequential leukaphereses, such as every 2 or 3 days. It was found that there is after the first 4 leukaphereses always an increase in the blood CFU-C concentration as compared to the previous CFC. Thus, when leukaphereses are

being performed sequentially, the CFU-C yield can be increased. In one series of studies, the yield of a fourth leukapheresis was about 4 times as high as of the first collection. A third approach was used which is to prolong the CFC up to 24 hours. It was found, that the number of CFU-C that can be collected is increased, as the time of CFC is prolonged. However, due to a reduction in the number of circulating platelets that are removed in the CFC process, the prolongation of CFC has other limiting factors. The second question was related to the development of cryopreservation procedures for large fluid volumes containing hemopoietic progenitor cells. It was found that the viability of mononuclear cells including CFU-C was about 80 % or better when compared to fresh cells regardless whether freezing and thawing was performed in ampoules or in plastic bags. It was necessary to use DMSO as a cryoprotective agent in a final concentration of about 10 % in the cell suspension to be frozen and to control the rate of freezing, especially during the transition phase. Furthermore, when plastic bags are being used, the thickness of the bag has to be constant which is attained by placing it between 2 copper plates. Using plastic bags as a containment, large volumes of cell suspensions amounting up to 350 ml could be cryopreserved and stored for 7 to 27 months before their transfusion into lethally irradiated dogs. The recovery of mononuclear leukocytes after 27 months of storage was 86.5 %, that of CFU-C about 90 % per leukapheresis.

The third question was related to the problem of the possible relationship between the number of transfused autologous mononuclear blood leukocytes among them hemopoietic progenitor cells measured as CFU-C and the short and long term hemopoietic regeneration of a 1200 R whole body x-irradiated recipient dog. The blood cells were obtained by repeated continuous flow centrifugations after dextran-sulfate mobilization. All cells were cryopreserved before transfusion. If the tenth day is used as an endpoint to measure the short term cell dose - marrow regeneration relationship it was established that there is a clearcut cell dose dependancy of the degree of bone marrow regeneration and blood cell recovery. The more cells and in particular the more CFU-C are given, the more rapid is the bone marrow regeneration. At doses of 1.5 - 2.0 x 10^5 CFU-C per kg body weight, the marrow cellularity of the 1200 R irradiated recipient is back to normal. Lower cell doses such as 2 - 4 x 10^4 CFU-C per kg body weight also result

in hemopoietic regeneration but the time required for regeneration is prolonged. The follow-up of autologous transfused irradiated dogs indicated that the final return of blood cell values to normal will take up to 100 days. The dogs were then followed up to about 1000 days and it was found that the hemopoietic restauration was a permanent one. This was also true for the lymphatic system and the reactivity of the lymphocytes to PHA stimulation. Thus, it was concluded, that it is not only possible but feasible to collect hemopoietic progenitor cells from the peripheral blood, cryopreserve them and to restore with them the entire hemopoietic system of a lethally irradiated recipient in the autologous situation. It is obvious that this has interesting implications for human radiation protection research. Because, if the preclinical observations could be shown to be true also for man, then one could establish a "stem cell collection" for each person that might be subject to the risk of becoming overexposed to radiation or other cytotoxic agents.

The fourth question was related to the problem of the allogeneic use of hemopoietic stem- and progenitor cells from the blood for hematopoietic reconstitution of a whole body irradiated recipient. The special problem is of course the question as to the possibilities to avoid graft-versus-host-disease (gvhd). We used the approach as suggested first by van Bekkum and Dicke which is directed to "purify" out of the heterogeneous population of "mononuclear" cells that subpopulation endowed with a pluripotent hemopoietic potentiality. We used the discontinuous albumin gradient and found that "fraction 2" of this gradient may contain as many as one CFU-C per 20 mononuclear cells (MNC) as compared to about one CFU-C in 10 000 to 15 000 MNC in a normal blood cell suspension. This can only be attained by placing a CFU-C enriched cell population on the gradient which can be obtained -in dogs- by collecting high CFU-C numbers from the blood by DS-mobilisation and repeated leukaphereses. If cells of fraction 2 are used for hemopoietic reconstitution of a lethally whole body irradiated dog it was found, that no lethal gvhd occurred (using DLA matched, MLC-negative donor recipient relationship and opposite sex combination for proof of engraftment). In these dogs, no immunsuppressive therapy was used after allogeneic cell transfusion. In contrast, cell fractions with a sufficient CFU-C content but a high T- or B-cell contamination (fractions 3 and 4) resulted in a very severe gvhd so that all animals died within about 40 days. The serum

transaminase levels could well be used to monitor the presence and progression of the gvhd. The dogs that survived the allogeneic blood CFU-C transfusion all had obtained the "purified" CFU-C population "fraction 2". A complete hemopoietic reconstitution was obtained that persisted until the end of the experimental observation period (several hundred days). By means of sex-chromosome-marker evidence was obtained that a chimerism existed. Of passing interest was the observation, that perviously blood CFU-C transfused allogeneic recipients treated with unseparated MNC and with Methotrexate after whole body irradiation showed all a complete chimerism. In contrast in the animals given separated allogeneic cells and no methotrexate post-transplantation therapy a split chimerism was observed. This is in accordance with the knowledge, that even a whole body exposure with 1200 R does not eliminate all lymphatic stem-/progenitor-cells so that some may indeed be able to proliferate. However, this split chimerism nevertheless was tolerated by the recipient and no late rejection or chronic gvhd was observed.

Finally, the question of the late consequences of whole body exposure in the lethal range and of autologous as well as allogeneic blood mononuclear cell transfusion was intensely studied. In all dogs that survived 1200 R whole body x-irradiation, typical non-neoplastic late effects lesions were observed. Of particular interest was the observation of pancreatic cirrhosis as well as fibrotic lesions in lungs, kidneys and bone marrow. Minor radiation induced lesions were found in other organs. These lesions were more severe in animals treated with cytotoxic substances (post-transplantation immunosuppression) given in addition to whole body irradiation than in those animals given irradiation only. Although such lesions were observed in principle, one might ask the question why they were not regularly observed. The incidence of organ fibrosis was less than one might expect. Therefore, the question may be asked whether the reestablishment of macrophage populations after stem cell transfusion might be relevant for this observation. Therefore, in future studies, the role of complete or incomplete hemopoietic recovery or its manipulation for the development of radiation induced late effects should be intensely studied.

Ergebnisse des Projektes/Results of Project No. 3

Leiter des Projektes und wissenschaftliche Mitarbeiter (1976-1980):

Proffs. Drs. T.M. Fliedner and H. Heimpel in association with Drs. R. Arnold, F. Carbonell, S.F. Goldmann, M. Haen, H. Gerhartz, M. Körbling, B. Kubanek, E. Kurrle, P. Lohrmann, H. Pflieger, W. Schreml

Titel des Projektes/Title of Project:

Treatment of hemopoietic failure in man by means of autologous and allogeneic stem cell transfusion such as seen after radiation exposure.

Darstellung der Ergebnisse/ Presentation of results:

In an attempt to investigate whether the concepts developed in the preclinical canine model for a "blood stem cell bank" can be transferred to the clinical level for hemopoietic reconstitution of patients with bone marrow failure a number of studies were performed during 1976 and 1980 and yielded the following results. As indicated already in Project No. 1, human blood contains a small but significant number of granulocytic as well as erythropoietic progenitor cells which is in the order of 200 and 900 CFU-C and BFU-E per ml,respectively. Using the "diffusion chamber method" it was possible to show, that CFU-C retain a certain degree of replicative capacity. The fact that mononuclear cells (MNC) from human blood when cultured in a diffusion chamber intraperitoneally in mice can give rise to all hematopoietic cell lineages is interesting evidence for the pluripotency of some of the blood mononuclear cells. The CFU-C can be collected from human blood by means of leukocytapheresis (CFC). 35 volunteers were studied. One leukocytapheresis may allow one to collect close to 9×10^5 CFU-C within 4 hours or 12×10^9 MNC. This yield can be increased by repeating the CFC every 2 to 3 days. It was found, that the third CFC performed resulted in the collection of about twice as many CFU-C as from the first CFC. Due to the fact that there was a CFU-C overshoot in the blood lasting for several days after the last CFC it is assumed, that this phenomenon should be utilized in future studies to improve even more the CFU-C collection yield. In 3 successive leukapheresis in man, one is able to collect an average of 23.0×10^5 CFU-C from the blood. If 5×10^5 CFU-C are necessary per kg body weight to obtain a hemopoietic restauration (as was found for bone marrow transplantation) then one would need approx. 5 leukapheresis (without mobilizing drugs!) to collect enough cells for a hemopoietic reconstitution in the autologous situation. In the course of the last 5 years, a closed plastic bag system was developed and tested for the collection, cryopreservation, thawing and

transfusing mononuclear cells collected from human blood. It was found in 20 separate CFC-runs, that the mean number of CFU-C collected was $7.5 \pm 4.4 \times 10^5$. After cryopreservation in plastic bags, the recovery was excellent, showing a 95.7 % value. Thus, the principle possibility of a "blood stem cell bank" in man appears to be established. How can one improve the yield of MNC and of CFU-C collection? When patients with suitable neoplastic diseases were given high dose cyclophosphamid therapy, a marked CFU-C overshoot in the blood was observed with a peak 14 to 16 days after the cyclophosphamide application. The overshoot was measured to be in the order of a factor of 10 - 20 as compared to the pretreatment value. Such a pattern was observed to occur after each course of administration of cyclophosphamide and adriamycin. Thus, the question arose whether this type of overshoot might be used in collecting CFU-C from patients with neoplastic disorders in large numbers in order to return them to the patient after cytocidal chemo- and/or radiation exposure. So far, this question was studied in a canine model. Mononuclear leukocytes including CFU-C were collected from dogs treated with a single dose of cyclophosphamide 10 to 13 days later by means of CFC and cryopreserved. Such a single CFC yielded about 50×10^5 CFU-C. Between 2 and 5 months later, these MNC incl. CFU-C were thawed and transfused in the autologous situation to the dog given 1200 rad whole body irradiation. Between 20,000 and 100,000 CFU-C per kg body weight were used, a dose that was proven to be sufficient when given in the autologous situation and taken from an untreated dog under the same radiation exposure conditions. It was found, that at either dose of CFU-C transfused, a regeneration of the bone marrow could be obtained. However, there was a remarkable delay in the bone marrow repopulation in those dogs that received between 20,000 and 60,000 CFU-C collected after pretreatment with cyclophosphamide when compared to CFU-C collected from normal dogs. From these findings it appears debatable whether the removal of MNC including CFU-C from donors pretreated with cytotoxic agents will yield a number of stem cells large enough to be still capable of unlimited replicative potential as is necessary for the complete restauration of a radiation induced hemopoietic aplasia.

Of considerable importance were the studies in which patients were given total body irradiation, followed by bone marrow transplantation. A leukemic patient was given 850 rad of whole body irradiation using a linear accele-

rator followed by a bone marrow transfusion (2.14 x 10^8 cells per kg body weight) derived from his twin brother. The granulocyte count started to rise after about 10 - 11 days. At the time of discharge, the blood cell counts had returned to normal. This patient was of importance because of the chromosomal findings. The chromosome pattern was normal with respect to a leukemic cell clone that was present before irradiation and twin brother bone marrow transfusion. Thus, there was evidence for a successful engraftment. However, some metaphases of PHA stimulated lymphocytes were present showing radiation induced chromosomal aberrations. This indicates once again, that 850 rad are not sufficient to eliminate the lymphocytic system of the recipient. This may well have consequences for radiation accidents in which the total exposure dose is not known. Here it may well be necessary to "condition" such a patient with an immunosuppressive regimen.

In the human situation, blood derived mononuclear cells containing hemopoietic stem cells cannot be used for allogeneic reconstitution of hemopoiesis until more evidence is obtained with respect to the avoidance of gvhd. In dogs, the "purification" of MNC suspensions has been shown to be successful if the concentration of CFU-C became very high and the T- and B-cell contamination very low. Thus, studies were initiated to examine the possibility of obtaining - by means of the discontinuous gradient technology - a MNC-suspension rich in CFU-C and poor with respect to T- and B-cells. All experiments utilizing the albumin density gradient remained unsatisfactory. More recently, the use of "Percoll", a colloidal suspension of poly-Vinyl-Pyrrolidone (PVP) coated Silica particles, was attempted. It was found, that it is possible to obtain MNC fractions containing about 80 % of the original CFU-C but only 2 % of the original lymphocytes. If these results can be reproduced in future experiments, there will then be an excellent perspective for obtaining stem cells in man from the peripheral blood in a purified form by first collecting MNC by means of CFC, separating progenitor cells from immunocompetent cells and by large fluid volume cryopreservation. Open problems are the mobilization of stem cells into the peripheral blood using non-cytotoxic approaches and the in-vitro characterization of pluripotency of cells in the MNC-suspension obtained from human blood.

Ergebnisse des Projektes/Results of Project No. 4

Leiter des Projektes und wissenschaftliche Mitarbeiter (1976-1980):

Proffs. Drs. T.M. Fliedner and H. Heimpel in association with Drs. R. Arnold, W. Calvo, E.B. Harriss, H. Heit, W. Heit, E. Kurrle and H. Pflieger

Titel des Projektes/Title of Project:

Bacterial decontamination of mice and man: survival after whole body irradiation and stem cell transfusion in relation to the microbial flora.

Darstellung der Ergebnisse/Presentation of results:

The project examines the hypothesis - proposed several years ago - that the graft-versus-host-disease (gvhd) seen in several mammalian species including man after allogeneic bone marrow transplantation is related to the presence or absence of a microbial flora. While it was established that germfree mice tolerate an allogeneic bone marrow transfusion without dying from a gvhd, the exact relationship between the bacterial contamination and the actual state of the microbial flora remained nuclear. In this report, the results of studies in mice as well as in patients are summarized in which the questions were asked whether and why mice having been completely or selectively decontaminated survive the consequences of a lethal whole body irradiation and allogeneic bone marrow transplantation and whether one can decontaminate patients in such a way that it may influence the gvhd after allogeneic bone marrow transplantation.

Previous studies have clearly ruled out the prognostic value of the gnotobiotic state for lethally irradiated mice to survive allogeneic bone marrow grafting and gvhd. In contrast to a 100 % death rate for recipients manitained in a conventional environment, more than 80 % of the germfree or decontaminated recipients recovered from transient gvhd and established hemopoietic and lymphopoietic long time chimerism.

In 1980 studies were continued to determine the role of the bacterial flora for the prognosis of delayed gvhd. Previous results could be confirmed that germfree allografted bone marrow recipients could be associated with an anaerobic or aerobic flora or both as early as 8 days after and 8 weeks before transplantation. No animal died, even when aerobic microorganisms from septic human individuals were used in combination with intestinal anaerobes. From the experimental data available no single bacteria could be identified as a significant risk for a lethal gvhd in germfree

allochimeras, thus indicating that a bad prognosis of allogeneic marrow recipients may be related to more complex interactions between the graft and a host associated with a microflora that is composed of a variety of different bacterial individuals.

Hempoietic recovery in the allogeneic host was confirmed to follow a similar pattern compared to isogeneically grafted bone marrow recipients. The femoral content of CFU-S and CFU-C as well as the peripheral blood counts turned back to control values within 8 - 10 weeks after lethal radiation exposure (800 rad TBI) and bone marrow transplantation (10^7 cells). Furthermore chimeric bone marrow (week 10 - 20 after engraftment) showed a normal repopulating potential when passaged into a second isogeneic or allogeneic host. None of these recipients died by bone marrow failure or gvhd, even when 3×10^7 spleen cells were added, thus confirming the concept of aquired immunotolerance of the graft to occur in allogeneic bone marrow recipients surviving gvhd.

Acute gvhd could not been overcome by decontaminated or germfree recipients. All animals grafted with 10^7 BMC and 3×10^7 spleen cells died within 20 days. Based on these observation the following hypotheses were examined:

1. The risk for lethal gvhd correlates with the number of immunocompetent cells present in the allogeneic bone marrow graft.
2. The gvhd is T-lymphocyte dependent
3. Gnotobiotic recipients survive an increased number of allogeneic immunocompetent cells compared to conventional controls.

The results of representative experiments can be summarized as follows: Conventional and germfree allogeneic recipients with radiation induced bone marrow failure died within 3 weeks when 3×10^7 spleen cells and 1×10^7 bone marrow cells were grafted. A subacute but lethal course of gvhd occured in conventional and in germfree allogeneic bone marrow chimeras which received in addition 3×10^6 or 10^6 spleen cells. Practically all germfree allogeneic recipients survived which were given an additional spleen cell graft of 10^5 or 3×10^5 cells per animal.

Anti-Theta-Globulin (ATG) did abolish lethal gvhd in germfree recipients

grafted with BMC (10^7) and 3 x 10^6 spleen cells but failed to compensate for the lethal effect of 3 x 10^7 spleen cells.

From these studies, the state of our knowledge can be summarized as follows: No unifying concept can be offered as yet to explain the improved prognosis of gnotobiotic in contrast to conventional allogeneic bone marrow recipients. The risk of bacterial infection during the phase of regenerating hemopoiesis may obviously enhance the mortality risk due to gvhd in conventional animals. However, it indicates the need for increased numbers of immunocompetent cells to induce lethal gvhd in gnotobiotic recipients. The improved chance to survive gvhd and establish immunotolerance may be ascribed to quantitative or qualitative differences in the presentation of H-2 related antigens.

The inappropriate effect of ATG to suppress lethal gvhd due to 3 x 10^7 spleen cells furthermore depicts a new problem in allogeneic bone marrow grafting indicating that other than post thymus T-lymphocytes may be involved in the induction of gvhd.

To further investigate there actual questions, a new experimental design has to be formulated, which will be the subject of another conceptional approach.

The studies in human beings were performed in accordance with the clinical protocols of the EORTC-Gnotobiotic Project Group. During the first part of the contract period (1976-1980) many studies were performed in human beings treated in a plastic isolation bed system to attempt a complete microbial decontamination by means of administration of a mixture of antibiotics, most of them non-resorbable. Although it was consistently shown that the feces became free from bacteria, it was also found that it is difficult to achieve a complete removal of the microbial flora of the oropharynx. However, a "gnotobiotic state" was certainly achieved and resulted in a reduction of the incidence of bacterial infection in spite of a low blood granulocyte concentration. However, in the second part of the contract period, a novel approach was used to achieve a sufficient degree of control of the microbial flora. A "selective" decontamination of patients is being attempted and has the goal to eliminate pathologenic or potentially patho-

genic bacteria with the simultaneous protection of the generally apathogenic anaerobic microflora. This selectivity is largely responsible for "colonization resistance" and protects from the colonization with new bacteria. Patients with acute leukemia were treated with Polymyxin B, Nadidixinsäure, Neomycin und Nystatin. The results indicate that gram negative bacteria can be readily eliminated. Anaerobic bacteria can be demonstrated continuously in the stools. In about half of the patients no bacterial infections were observed in spite of the severe granulocyte depression. The other patients showed only minor infection of the upper respiratory tract. However, there was still an occasional death from septicemia.

In conclusion, attempts to transfer the results of experimental studies to clinical applications are being continued. They all indicate the importance of the composition of the microbial flora for mortality after whole body irradiation (or cytotoxic therapy) and allogeneic bone marrow transplantation due to infection (granulocytopenia) and/or graft-versus-host-disease. While it appears likely that it is not essential in mice nor in man to achieve a complete bacterial decontamination for improval of survival rates, there is evidence that a selective decontamination results in an elimination of certain elements of the aerobic flora and allows the anaerobic flora to improve the colonization resistence. It remains to be established in what way the microbial flora influences the manifestation and severity of graft-versus-host-disease after allogeneic bone marrow transplantation.

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Contractor of the Commission	:	Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek TNO
Contract No.	:	198-76-1 BIO N
Head of research teams	:	Prof.Dr. D.W. van Bekkum
General subject of contract	:	Bone marrow transplantation

GENERAL INTRODUCTION

The bone marrow transplantation programme in Rijswijk comprises research in rodents which is directed at the fundamental aspects of bone marrow transplantation, and preclinical experiments with monkeys and dogs. The preclinical studies were the subject of the contract in the 1976-1980 Radiation Protection Programme. The major research objective was identification of the factors that permit a successful bone marrow transplantation for recipients who do not have an available brother or sister that is identical for the major histocompatibility complex (MHC) to serve as a marrow donor. During the past 4 years, major progress has been made in identifying these factors.

For a successful allogeneic bone marrow transplantation a double immunological barrier has to be overcome: 1) the so-called host-versus-graft (HvG) reaction, resulting in rejection of the graft, and 2) the graft-versus-host (GvH) reaction, which is an immunological attack on the host launched by lymphocytes which are present in or originate from the graft. The identified major factors that determine acceptance of the graft in the host and the severity of the resulting GvH reaction are listed in the following table.

TABLE I

Major factors determining

Acceptance or "take" of the graft	Type and severity of graft-versus-host disease (GvHD)
1. the degree of immunogenetic disparity, especially with regard to the products of the major histocompatibility complex, or MHC	
2. the composition of the graft: the ratio of haemopoietic stem cells to immunocompetent T-lymphocytes	
3. the number of cells transplanted	
4. preparation ("conditioning") of the recipient	4. sex of the donor
5. prior sensitization of the host, e.g. by transfusions	5. composition of the gastro-intestinal microflora of the host

It is shown in this report that:

1) these factors are interdependent;

2) Apart from histocompatibility, measures taken to mitigate GvHD tended to decrease the frequency of takes; vice-versa, measures taken to promote engraftment appeared to promote the development of GvHD as well. The practical implication of this "reciprocal interference" of HvG and GvH reactions is, that patients will only benefit from measures taken to prevent GvHD if simultaneously measures are taken to promote engraftment.

3) Partially mismatched and probably also completely mismatched donors can be used successfully for bone marrow transplantation by the judicious application of the other factors that beneficially influence the acceptance of the graft and the development of GvHD.

Schematically, the subject matter of this contract can be subdivided into three projects. For the purpose of this report we shall therefore discuss them separately and in the following order:

1. donor selection in rhesus monkeys
2. donor selection in dogs
3. prevention of infection, and mitigation of GvHD by gastrointestinal decontamination.

The rhesus monkey and dog projects are largely complementary. Rhesus monkeys are mainly used to study the prevention of GvHD in mismatched donor/recipient combinations, and relatively little work has been done with littermate combinations. The large litters of dogs made this species especially suitable for studies in MHC-identical sibling combinations. In this species the mismatched combinations were mainly used to study conditions that promote engraftment.

Title of project No. 1 : Donor selection in rhesus monkeys

Head of project and scientific staff : Dr. G. Wagemaker, Dr. H.M. Vriesendorp, Prof.Dr. D.W. van Bekkum, E.P. Walma, P.J. Heidt, Prof.Dr. H. Balner.

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1. Introduction

One of the important issues in bone marrow transplantation is the selection of suitable donors for those recipients that lack a sibling donor identical for the MHC, particularly with regard to the prevention of GvHD. Previous work in rodents and limited experience in rhesus monkeys showed that major mitigating effects on GvHD are to be expected from immunogenetic compatibility and selective elimination of immunocompetent cells from the graft. The relative importance of each of these measures, however, has never been investigated in an outbred species like man. The preclinical experiments with rhesus monkeys aimed to close this gap in our knowledge and to provide an experimental basis for the development of a donor selection policy in man.

2. Histocompatibility research

As already indicated, the cell membrane antigens controlled by the MHC play a most important role in histocompatibility. For that reason MHC defined antigens were the primary subject of our research in the past years. Basically, there are 3 classes of relevant MHC products. Class I are the serologically defined A and B locus antigens, also designated as SD antigens. Class II comprises the so-called Ia antigens; they can be subdivided into those defined by the D or major MLC* locus and those controlled by the D-Related or DR locus. It is still not known whether D and DR locus antigens are identical. Finally, class III products are a number of complement factors which are probably of lesser importance with regard to histocompatibility.

By 1976 our knowledge of the A and B locus antigens (class I or SD) was already rather advanced. In the course of the past 4 years a few new SD antigens of rhesus monkeys were discovered, the serological identification of several established antigens was improved and certain chemical characteristics of SD antigens defined.

In contrast, in 1976 our knowledge of the biologically very important Ia or class II antigens was still rudimentary. Hence, maximal attention was paid to this subject in the course of this contract period. Chronologically, the first series of allelic Ia antigens was described in 1976. Some of the biological characteristics of Ia antigens were also reported in that period. Subsequently, several D locus antigens were identified by means of cellular techniques (with so-called homozygous typing cells, in mixed lymphocyte cultures). Then the close association between Ia and D locus antigens was

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* antigens with an overriding influence on lymphocyte reactivity in mixed cultures.

established and the major locus of Ia antigens was henceforth designed as "DR". The close association made it possible to select D antigen identical unrelated individuals (which are nonresponsive in MLC) by matching for the serologically defined DR antigens. Finally, investigations were carried out to identify tissue antigens controlled by loci other than the A, B and D/DR. By 1980, solid evidence was available that there are indeed such antigens. However, their relevance to histocompatibility in skin-, organ- and bone marrow transplantation, is still uncertain.

3. Effect of donor selection on take of bone marrow stem cell concentrates and resulting GvHD

Using conventional barrier nursing a relatively large series of monkeys has been transplanted. These included identical sibling donors, (half-)sib donors which share one MHC haplotype with the recipient, sibling donors which share one haplotype and are, in addition, phenotypically identical for D and DR loci, unrelated donor recipient pairs matched for products of the A and B loci of the MHC, unrelated D/DR-matched combinations and unrelated mismatched combinations. Table II summarizes results with regard to the take frequency in different donor/recipient combinations under various experimental conditions.

TABLE II

TAKE FREQUENCY IN DIFFERENT DONOR/RECIPIENT COMBINATIONS FOR UNFRACTIONATED VERSUS STEM CELL ENRICHED GRAFTS[1] AND FOR TWO CONDITIONING REGIMENS

Donor/recipient combination			Number of takes/total		
RhLA		Family	Unfract.	stem cell grafts	
A/B	D/DR	relationship	grafts 8.5 Gy X-rays	8.5 Gy X-rays	2 x 7.0 Gy[2] X-rays
=	=	siblings	6/6	5/5	
≠	=	haploidentical siblings		2/2	3/3
≠	≠	haploidentical half-siblings		5/8	2/2
=	≠	none	4/4	9/10	
≠	=	none		3/3	1/1
≠	≠	none	7/7	5/14	5/5

[1] stem-cell-enriched, lymphocyte-depleted fractions, prepared by discontinuous albumin density gradient centrifugation

[2] separated by 3 days

The table shows a diminished takeability of purified stem cell concentrates as compared to unfractionated bone marrow in unrelated MHC mismatched combinations. If not corrected, this phenomenon would seriously restrict the clinical usefulness of such concentrates. Raising the single TBI (total body irradiation) exposure to 9.0 Gy did not decrease the incidence of take failures in mismatched unrelated donor/recipient combinations (1 take out of 3 transplantations). Restoration of the original bone marrow composition by adding irradiated non-stem cell fractions was also ineffective (2/6). In a follow-up study a split TBI (2 x 7.0 Gy, separated by 72 h, see project 2) was found to be a highly effective conditioning regimen for mismatched unrelated and haploidentical half-sibling donors (Table II).

Table III gives the frequencies of mortality due to GvHD and the median survival times in different donor/recipient combinations.

TABLE III

MORTALITY DUE TO GvHD AND MEDIAN SURVIVAL TIME IN DIFFERENT DONOR/RECIPIENT COMBINATIONS FOR UNFRACTIONATED VERSUS STEM-CELL-ENRICHED GRAFTS

Donor/recipient combination			Mortality due to GvHD			
RhLA		Family	Unfractionated bone marrow		Stem cell grafts	
A/B	D/DR	relationship	Frequency	Median surv. (days)	Frequency	Median surv. (days)
=	=	siblings	5/6	22	0/5	> 2 years
≠	=	haploidentical siblings			4/5	25
≠	≠	haploidentical half-siblings			7/7	28
=	≠	none	4/4	15	5/9	50
≠	=	none			4/4	20
≠	≠	none	7/7	12	10/10	24

It is shown that among these various groups the identical sibling combinations were outstanding in that no take failures of the stem cell grafts occurred and no lethal GvH reactions were seen. This series of transplantations resulted in long-term surviving chimaeras without evidence of immunodeficiency. If, however, unfractionated grafts were given, the majority of these monkeys died within 1 month from moderate to severe GvHD.

The stem cell grafts from haploidentical (half-)sib donors resulted in takes in only half of the cases, and the occurrence of delayed GvHD resulted in a median survival which did not differ from that of the control group of unrelated mismatched donor/recipient combinations. Similar results with respect to GvHD were obtained in both related and unrelated donor/recipient combinations matched for D-locus products.

The incidence and severity of delayed type GvHD in monkeys which had received stem cell grafts of unrelated donors matched for A and B locus products was significantly lower than in the unrelated mismatched donor/recipient group, resulting in a threefold longer mean and a twofold longer median survival time. These monkeys, however, died from infectious complications, presumably on the basis of prolonged immunodeficiency due to subclinical chronic GvH reactions. Takeability of the stem cell grafts in this group of monkeys was excellent. Grafting unfractionated marrow in this donor/recipient combination resulted in rapid death dur to acute GvHD.

These results stress the importance of matching for A and B locus products of the MHC with respect to both takeability of stem cell concentrates and mitigation of resulting GvHD, and invalidate the idea that matching for D locus products beneficially influences GvHD in the clinical situation. It is further shown that lymphocyte-depletion of the graft completely prevents GvHD in MHC-identical sibling donor/recipient combinations and confirmed that in (partially) mismatched combinations the "acute" type of GvHD seen after whole bone marrow transplantation can be changed consistently into "delayed" type GvHD by the use of stem cells. The advantage of a close MHC match with respect to mitigation of GvHD appears to be optimally expressed if the number of lymphocytes present in the graft is decreased. The use of lymphocyte-depleted stem cell concentrates, however, increases the risk of take failure. Successful engraftment of stem cell concentrates in mismatched donor/recipient combinations required, therefore, an improved conditioning regimen.

Title of project No. 2 : Donor selection in dogs

Head of project and scientific staff : Dr. H.M. Vriesendorp, E.P. Walma, Dr. G. Wagemaker, Prof.Dr. D.W. van Bekkum

1. Introduction

Clinical allogeneic bone marrow transplantation is almost completely restricted to the most favourable donor/recipient combination, the MHC identical brother or sister. Unfortunately, only 30% of the recipients have such a donor available. However, a sibling which is mismatched for one MHC-haplotype is available in about 50% of the recipients. The large litters of dogs makes this species especially suitable to study bone marrow transplantation in related donor/recipient combinations. Consequently, the dog model was restricted to MHC-identical and -haploidentical sibling transplantation.

2. Takeability of grafts

Total body irradiation (TBI) is the most effective single agent for preparing recipients for bone marrow transplantation. The optimum TBI dose was determined for bone marrow cells of a sibling donor that is identical for the MHC, i.e. a single exposure of 5.0 Gy (300 kV X-rays, dose rate 0.16 Gy/min HVL 3 mm Cu). A single TBI exposure even given at maximum tolerated dose (8.5 Gy) is insufficient for MHC mismatched marrow. A split TBI (2 x 6.0 Gy, separated by 72 h) was found to be an effective method without undue toxicity for 1 MHC haplotype different donors (Table IV).

TABLE IV

TAKEABILITY OF WHOLE BONE MARROW GRAFTS AND LYMPHOCYTE-DEPLETED STEM CELL CONCENTRATES* USING VARIOUS CONDITIONING REGIMENS IN DOGS

Conditioning TBI (Gy X-rays)	Donor/recipient combination	Graft	Takes/total
5.0	identical siblings	bone marrow	5/5
7.5		bone marrow	20/20
7.5		stem cells	2/5
2 x 6.0 (72 h interval)		stem cells	8/8
2 x 4.5 (24 h interval)		stem cells	4/4
2 x 4.5 (72 h interval)		stem cells	2/4
7.5	haploidentical sibs	bone marrow	0/5
2 x 6.0 (72 h interval)		bone marrow	5/5
2 x 7.0 (72 h interval)		stem cells	0/4

* prepared by discontinuous albumin density centrifugation.

Two fractions of TBI, 6.0 Gy X-rays each, separated by a 72 h interval resulted in a 100% engraftment of MHC-identical stem cells. The gastrointestinal toxicity that we observed in animals that received this high dose split TBI was low. The late toxicity of the high dose split TBI (+ bone marrow transplantation), i.e. pancreas pathology, recurrent pneumonia and wasting, however, prevents the application in humans.

We are now investigating the immunosuppressive potency of a low dose split TBI regimen (2 x 4.5 Gy, 24 or 72 h interval). With this lower dose split TBI regimen, engraftment was obtained in the majority of the recipients that received MHC-identical stem cells. This low dose split TBI group will be extended because of its great value for clinical application.

2. Graft-versus-host disease (GvHD)

In MHC-identical dog combinations, the addition of increasing numbers of donor lymphocytes to the bone marrow cell suspension causes a dose dependent increase in severity and incidence of GvHD. With 3 x 10^8 donor lymphocytes. kg-1 body weight recipient a 100% GvHD incidence is reached. A genetic analysis of the obtained results indicates that clinical symptoms of many different minor histocompatibility systems that control GvHD become apparent if T-lymphocytes are present in appreciable amounts (as in primate bone marrow). In view of the numerous minor histocompatibility systems involved and the well-known difficulties in obtaining reagents for a prospective identification of antigens of such systems and the limited availability of human MHC-identical sibling donors, the most realistic approach to control GvHD in such combinations in man appears to be the avoidance/elimination of donor lymphocytes in the graft, rather than attempts at donor selection. This was directly confirmed in the rhesus monkey model.

In view of the growing awareness on how to prevent or control GvHD in MHC-identical siblings, the program was extended to include a new donor category, the 1 MHC mismatched family donors. Almost every human patient will have such a donor (father, mother, 50% of the siblings), in contrast to only one in three that has a MHC identical sibling. The preliminary experience with suppressing the HvG reaction against such donors has been favourable (see 1.2.). Untreated, the median survival of such animals is limited to approximately 50 days. Death is due to GvHD. This is considerably worse than MHC-identical combinations (indefinite survival), but about twice as good as unrelated or 2 haplotypes different combinations (MST $\approx$ 25 days). Further experimentation will concentrate on the application of principles that were effective in suppressing GvHD in MHC-identical combinations (i.e. lymphocyte-depletion of the grafts and gastrointestinal decontamination of the recipients). So far, however, the takeability problems of stem cell grafts in these mismatched combination (Table IV) appeared the major stumbling-block.

Title of project No. 3 : Prevention of infection and mitigation of GvHD by gastrointestinal decontamination

Head of project and : P.J. Heidt, Dr. H.M. Vriesendorp, E.P. Walma,
scientific staff : Dr. G. Wagemaker, Prof.Dr. D.W. van Bekkum

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Fundamental work in rodents indicates that certain bacteria of the microflora belonging to the Enterobacteriaceae activate allogeneic donor lymphocytes in starting and possibly maintaining an immunological reaction against intestinal epithelium. In mice, GvHD can be completely prevented if the recipients are germfree or have been subjected to prior gastrointestinal decontamination (GID). The beneficial effect of GID on mortality due to GvHD does not occur, however, if immunocompetent T-lymphocytes from the spleen are added to the bone marrow graft. A recent study unequivocally showed that in H2-incompatible donor/recipient combinations even low numbers of lymphocytes added to the bone marrow graft have a detrimental effect on the development of GvHD in germfree mice.

In dogs, GvHD is rare in recipients of MHC-identical sibling bone marrow, and never fatal. The GvHD incidence and mortality can considerably be increased, however, if donor lymphocyte cells are added to the graft. GID proved to be effective in preventing GvHD in this donor/recipient combination, if the number of added lymph node cells was low (10^8/kg) but failed if higher numbers (3×10^8/kg) were added. This suggests that relatively low numbers of lymphocytes can be tolerated in MHC-identical sibling bone marrow grafts without affecting the beneficial effect of GID on GvHD.

Also in the dog model, GID was performed in two modifications: 1) total GID (GIDT), where all micro-organisms with the exception of viruses are removed, and 2) selective GID (GIDS), where only gramnegative, aerobic bacteriaceae and fungi and yeast are eliminated. Only GIDT was effective in preventing endogenous infections after TBI. It was more difficult to maintain GIDT than GIDS. The success percentages were respectively 25 and 100%. The endogenous infections in dogs treated with GIDS were less severe than in conventionally treated animals. GIDS had a beneficial effect on acute and delayed GvHD in MHC-identical donor/recipient pairs (bone marrow and additional lymph node cells). GIDS was discontinued at day 40 after TBI. In the second and third month after TBI, parasitic and viral infections were freqently observed. In contrast, conventional animals do not show these types of infection at this stage of the experiment. This might indicate a slower development of the donor immune system in GIDS treated animals. This possibility and eventually corrective measures (as infusion of donor lymphocytes) will be subjected to further study. In possible agreement with this hypothesis, leukocyte recovery was found to be slower in animals with GID and bone marrow transplantation than in conventional animals.

In monkeys, prevention of GvHD by GID was studied in recipients of stem cell concentrates from unrelated A and B locus antigens matched donors, haploidentical sibling donors and unrelated, mismatched donors. The former combination was selected because of its promising results in the conventional situation, the latter two because of their abundant availability in the human population. Pilot experiments with GIDS were complicated by an unacceptably high incidence of endogenous infections. Therefore, all further

monkeys were subjected to total gastrointestinal decontamination. So far, eight monkeys with sustained engraftment (out of 19 irradiated) have been completely evaluated. The take failure frequency, though high, was not significantly different from conventional controls. Seven of the monkeys with sustained engraftment, including two recipients of unrelated, mismatched stem cells, showed no or only lowest grade, transient GvH reactions. Five of these survived recontamination as stable chimaeras; the other two died, respectively from cytomegalovirus infection and from uncorrected electrolyte imbalances due to the long-standing diarrhoea which occurs in all decontaminated monkeys. One monkey died from GvHD, which is attributed to an early accidental colonisation of two Enterobacteriaceae species. Apart from one pneumonia, no evidence for severe immune deficiency was obtained in any donor/recipient combination. Three of these monkeys, notably including one that received unrelated, mismatched stem cells, currently survive for periods of more than 1 to 3 years after transplantation without further immunosuppressive therapy.

These results are extremely encouraging with regard to bone marrow transplantation of radiation victims who do not have an available MHC-identical brother or sister to serve as a marrow donor.

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RAPPORT D'ACTIVITE 1976-1980

Contractant de la Commission	:	UNIVERSITE LIBRE DE BRUXELLES
N° du contrat	:	161-76-1 B108
Chef du groupe de recherche	:	STRYCKMANS P.
Thème général du contrat	:	Human peripheral blood hemopoietic stem cell kinetics, migration, collection, preservation and transfusion after ionizing irradiation.

SUMMARY OF THE RESULTS OBTAINED DURING THE PROGRAM 1976-1980

1. Studies on autologous bone marrow transplantation (ABMT)
   - ABMT shortens the period of post-chemotherapy marrow aplasia
   - Bone marrow stem cells show a great capacity to recover after exposure to high doses of toxic chemical agents
2. Studies on peripheral blood CFU-GM stem cells
   - Their number increases during marrow recovery after aplasia
   - Their number increases in metastatic marrow invasion
   - Normally most CFU-GM, if not all, are out of cycle
   - They are less resistant than marrow CFU-GM to 4°C storage
   - They reenter the cell cycle during marrow recovery
3. Studies on preservation of marrow CFU-GM stem cells
   - More than 90% survive 4 days storage at 4°C
   - Storage at -196°C decreases their response to human placenta conditionned medium (HPCM)
4. Studies on the regulation of committed stem cells
   - Erythropoiesis in vitro is inhibited specifically by parathormone
   - Granulopoiesis is not affected by lactoferrin, in vitro
   - Granulopoiesis in vitro is inhibited or regulated by lymphocytes
   - The eosinophil stem cell can be specifically affected
5. Studies on mature granulocytes (PMN) transfusions
   - Blood PMN and marrow reserve PMN are cytochemically different
   - Normal and leukemic PMN inhibit equally granulopoiesis in vitro

Résultats du projet

Chef du projet et collaborateurs scientifiques : P. STYCKMANS, A. DELFORGE, M. MALARME, D. BRON, T. SPIRO, R. MONSIEUR, E. RONGE-COLLARD, L. DEBUSSCHER

Titre du projet : Mechanism of release, kinetics and morphologic features of hemopoietic stem cells in the blood during marrow regeneration.

I. AUTOLOGOUS BONE MARROW TRANSPLANTATION

We have evaluated the validity of this procedure for bone marrow reconstitution on human beings made deliberately aplastic. Patients with malignant brain glioma have an extremely poor prognosis and require very high doses of cancer chemotherapy. This type of chemotherapy being extremely myelotoxic can be given only if followed by a marrow reconstitution manoeuvre. Seven subjects received 3 times the usual dose of CCNU (nitrosourea) followed within a few days in 5 subjects by the transfusion of cryopreserved autologous marrow. All the patients recovered. The 5 who did receive autologous marrow recovered significantly faster than the other (9). This procedure of marrow reconstitution was used in other patients who also received intense chemotherapy before. Ten patients with malignant hemopathies showed total marrow recovery after very high doses of BCNU (600 mgr/m^2 BSA) and m-AMSA (1 gr/m^2 BSA) followed by the administration of cryopreserved autologous bone marrow (19). In 8 patients with small cell anaplastic tumor of the lung, intensive chemotherapy was followed by marrow recovery whether the patient received autologous marrow or not (21). These studies indicates (a) that the marrow capacity to recover after chemically induced aplasia is considerable and that irreversible aplasia is rarely obtained (b) that the demonstration of the efficacy of autologous stem cell graft on chemically induced marrow aplasia is thus difficult to obtain.

II. SEARCH FOR CONDITIONS WITH INCREASED LEVELS OF HEMOPOIETIC STEM CELLS COMMITTED TO GRANULO-MONOCYTOPOIESIS (CFU-GM)IN THE PERIPHERAL BLOOD

Pathological situations exist which are characterized by an increased number of circulating CFU-GM. One aim of the project was to discover procedures capable of inducing whenever necessary, increased levels of circulating CFU-GM.

a) Recovery from chemically induced marrow invasion. It was known that the phase of recovery following methotrexate-induced marrow depression and peripheral blood granulo-thrombocytopenia is accompanied by a transient but significant rise of circulating CFU-GM. We have observed that various cytotoxic drugs can elicit such a post-aplasia CFU-GM peak. The drugs tested were CCNU (chloroethyl-cyclohexyl nitrosourea), DDMP (diamino-dichlorophenyl methyl pyrimidine), phenylalamine mustard, and various drugs associations.

b) Methylprednisolone and ethiocholianolone, 2 agents known to increase the release of polymorphonuclear cells from the marrow into the blood, did not increase the release of CFU-GM in the peripheral blood of human volunteers.

c) Filtration leukopheresis and electric convulsive therapy were not found to be followed by an increase of CFU-GM in the peripheral blood.

d) Local gamma irradiation was tested in 5 subjects. They received as therapy a local irradiation by a gamma ray source daily at a total dose ranging from 2,000 to 4,000 R over a period of 2-4 weeks. The upper limit of normal blood CFU-GM (0-40/ml of blood) was not exceeded during the period of irradiation which lasted 2-5 weeks.

e) Severely anemic patients (4-8 gr hemoglobin/dl) were examined for the level of CFU-GM in their blood. It was normal in all cases and not influenced by rapid normalization of the hemoglobin level by red cell transfusion (20).

f) The presence of neoplastic cells in the bone marrow : out of 24 patients with neoplastic bone marrow invasion by a solid tumor, 6 presented an increased level of CFU-GM ($>$ 40/ml of blood). Such increase was highly associated ($P < 0.01$) with the presence in the blood of microscopically recognizable myeloid and/or erythroid cells (18).

III. CHARACTERIZATION OF THE PERIPHERAL BLOOD CFU-GM STEM CELLS

A. IN THE NORMAL HUMAN SUBJECTS

a) the fraction of peripheral blood CFU-GM in DNA synthesis. Eleven normal subjects had blood cells exposed for 30 minutes to a high concentration of tritiated thymidine in order to kill the CFU-GM in DNA synthesis. The growth in culture of the cells exposed and of the control cells makes it possible to calculate the fractions of CFU-GM in DNA synthesis. This value ranged from 0-10% It was in contrast with the 40% found for normal marrow CFU-GM.

b) Sensitivity to 4 days exposure at 4°C. Unfractionated peripheral blood cells of 13 normal individuals were maintained at 4°C for 4 days after the addition of 10% TC 199 medium. The CFU-GM recovery at day 4 was only 5% $\pm$ 2

SEM of the control at day 1. This was in sharp contrast with recovery (97% ± 8 SEM) obtained for bone marrow CFU-GM (10).

c) Sensitivity to chemical agents. Human marrow and peripheral blood have been exposed, in vitro, to various cytotoxic agents at 4 different dose levels and then assayed in the CFU-GM agar clonogenic system. A dose-related inhibition of CFU-GM growth was observed and an LD 90 could be determined. No significant difference of sensitivity could be observed between blood and marrow. This was observed after days exposure to cell-cycle-phase-specific agents or after 30 minutes exposure to non-phase-specific agents (11, 14).

B. DURING THE CFU-GM PEAK VALUES ACCOMPANYING MARROW RECOVERY

Several studies, including ours, indicate that differences exist between marrow and blood CFU-GM. They include : time necessary for colony formation in agar, resistance to 4°C storage, fraction of cells in DNA synthesis and proportion of eosinophilic colonies. The blood CFU-GM seen during bone marrow recovery (CFU-GM-R) were examined to determine whether they resemble the blood or the marrow CFU-GM. The fraction of blood CFU-GM in DNA synthesis reached the value seen for marrow CFU-GM. The colony type was mostly granulo-monocytic instead of eosinophilic as seen in normal blood. On the other hand, the sensitivity to storage at 4°C was that of the blood CFU-GM. It is not clear yet whether the blood CFU-GM-R is a stimulated blood CFU-GM or rather a marrow CFU-GM which is released in the blood only in very special conditions.

IV. PRESERVATION OF HUMAN CFU-GM

a) Storage at 4°C

Eighteen bone marrows collected from various cancer patients without hematological diseases and from normal subjects were added with 10% TC 199 medium and then stored 4 days at 4°C. Thereafter, they were tested for CFU-GM growth in the Pike and Robinson agar culture assay. The results of this study indicate that unfractionated bone marrow cells may be stored at 4°C with 97% ± 8 SEM recovery of the CFU-GM (10).

b) Cryopreservation at -190°C.

The bone marrow of 10 individuals has been cryopreserved at -190°C in liquid nitrogen for various periods of time. Before cryopreservation each sample showed identical numbers of CFU-C whether the colony stimulating activity (CSA) used was that of mononucleated blood cells or that of human placen-

tal conditioned medium (HPCM). After thawing, the samples were retested : their CFU-GM content, expressed in percentage of the original value, was 78 ± 13 SEM when stimulated by blood mononuclear cells, but only 6% ± 3,5 SEM when stimulated by HPCM. Additional experiments have shown that in the presence of HPCM, CFU-GM still respond to mononuclear cells in a dose-related fashion. These studies strongly suggest that on the CFU-GM surface different "receptors" exist for the CSA activity of the monocytes and that of HPCM, and that they are differently sensitive to low temperatures.

V. REGULATION OF THE HUMAN HEMOPOIETIC COMMITTED STEM CELLS

The study of the specific regulations of the various hemopoietic cell types may be of great importance for the study of marrow reconstitution by graft of marrow stem cells.

a) Erythropoiesis :

Parathormone was examined as a possible regulator of the growth of human erythroid progenitors (17). This was tested in vitro using increasing concentrations of PTH from 250 to 16,000 pg/ml of culture medium. The growth of CFU-C started to decrease at the concentration of 2,000 pg/ml and from then decreased significantly in a dose-related fashion to reach 30% of the control level at the concentration of 16,000 pg/ml. On the other hand the growth of CFU-GM was totally unsensitive to PTH whatever dose was used. PTH thus appears as a rather specific inhibitor of erythropoiesis which, when increased in vivo such as in renal insufficiency, could hamper the recovery of erythropoiesis.

b) Granulo-monocytopoiesis

- Lactoferrin (LF), a product of mature granulocytes, has been proposed by Broxmeyer et al. as a regulator of the marrow and blood CFU-GM growth. The conclusion of our extensive studies on LF is that this compound at concentrations ranging from 10^{-8} till 10^{-18} M does not inhibit granulopoiesis in vitro (15). Additional studies are conducted to exclude that the CFU-GM tested are already maximally inhibited due to the exposure either in vitro or in vivo to an unknown inhibition of granulopoiesis. The role of acidic isoferritin, a recently described inhibitor of granulopoiesis, is being examined in this perspective.
- The peripheral blood lymphocytes in our hands were constantly found to inhibit the growth of human CFU-GM. This was still seen when autologous CFU-GM

were plated over the blood lymphocytes. This was only partially suppressed by the irradiation (1000 R) of the lymphocytes. The role of a subclass of T-lymphocytes is not unlikely.
- Unknown CFU-GM regulators could also play an important role. So, the bone marrow of patients with malignant lymphoma contain less CFU-GM than normal. We demonstrated that this is due to a reversible inhibition of the CFU-GM growth. This is not due to (a) the infiltration of the marrow by lymphoma cells, (b) the presence of a circulating inhibitor (c) an increased production of prostaglandins E by the macrophages (d) an inhibiting activity of the total peripheral blood lymphocytes (16). The possible role of interferon, or acidic isoferritin or a subclass of T lymphocytes is presently investigated. The depression of CFU-GM seen in lymphoma could be due to the exageration of a normal regulatory mechanism.

c) Eosinophilopoiesis

These studies were conducted on the blood and marrow of a rather unique patient who had an isolated abnormality : a complete absence of blood and marrow mature and immature eosinophils for a period of 8 years as a consequence of a chemically induced agranulocytosis. Eosinophil CFU-C (CFU-EO) however were seen in normal amount in blood and marrow. No evidence was found for the destruction of mature, immature or even stem cell eosinophils or for the absence or destruction of an eosinophilopoietic factor (12). It is concluded that a stem cell defect affecting differentiation may be localized at the eosinophil committed stem cell level.

GRANULOCYTE TRANSFUSIONS :

1. Influence of the "age" of the blood PMN on their activity. Corticosteroids are often injected to granulocyte transfusion donors in order to increase the levels of polymorphonuclear cells (PMN) in their blood and thereby the number of PMN collected from their blood. These agents trigger the release of the marrow PMN reserve into the blood. The alkaline phosphatase activity of the PMN just released into the blood as a consequence of an injection of cortisol was found to be significantly lower than that of the PMN already circulating (6). This indicates that corticosteroids not only increases the number of circulating PMN but also modifies their age distribution and thereby their functional capacity.

2. Neoplastic patients often require prophylactic and/or therapeutic PMN transfusions against bacterial and/or mycotic infections. However, PMN have been shown to inhibit granulopoiesis in vitro and could thus be the source of a negative feedback regulator of granulopoiesis in vivo. Thus, PMN transfusion given to fight infection in aplastic patients could, at least theoretically, inhibit the recovery of granulopoiesis and therefore be contrindicated during the process of take of a transplanted autologous marrow. In chronic myeloid leukemia (CML) huge numbers of mature, functionning PMN are procuded. They are often transfused to other leukemic patients in aplasia. We examined in vitro whether CML-PMN do have less, inhibiting activity than normal PMN on granulopoiesis to see whether, in this respect, they could represent more suitable cells for transfusions to aplastic patients. In 10 successive experiments no difference could be observed between CML and normal PMN in their capacity to inhibit in vitro colony formation by an allogenic bone marrow (4, 7).

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Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung
mbH. München
Institut für Hämatologie

Nr. des Vertrages: 217/76 1 BIOD

Leiter der Forschungsgruppe:

Prof.Dr. S. Thierfelder

Allgemeines Thema des Vertrages:

Short-term effects of ionizing radiation on hemopoiesis and their treatment

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Allgemeine Darstellung der durchgeführten Arbeiten:

The program of this association concerned the study of immunological and radiation-induced changes in chimaeras. More basic investigations were performed in rodents, while preclinical studies were mostly undertaken in dogs. Systematic hematomorphologic studies of locally irradiated patients were undertaken using improved methods of biopsy and histology.
The relevance of some of the data regarding suppression of gvh with antibodies was tested in bone marrow transplantation of leukemic patients conditioned with high dose total body irradiation. In mice we could show that heterologous polyclonal and, more recently, monoclonal antibodies against T cells prevented graft-versus-host reactions in semiallogeneic H-2 incompatible F1 recipients. This suppression was shown to be associated with a full recovery of the immune functions of the chimaeras. Also in dogs anti-T antibodies suppressed gvh in 50% of semiallogeneic haploidentical dogs. So far it is not clear whether higher concentrations of anti-T or the addition of complement to the pretreatment will enhance our suppression of gvh in dogs. Histocompatibility testing,which was necessary for defining the degree of incompatibility in these bone marrow transplantations, was also studied for defining 11 lymphocyte determinants of the DLA-D locus using the mixed lymphocyte culture technique.
Since the survival of leukemic patients depends on the efficacy of therapeutic regimens including antileukemic whole body

irradiation to reduce or eradicate the leukemic cell clone, radiation tolerance was investigated. A varying difference of radiation toxicity for the hemopoietic and gastro-intestinal system was found. By lowering the dose rate and fractioning radiation total doses, over 20 Gy were compatible with survival if transplantation of autologous or DLA-identical bone marrow was applied thereafter.

Our approach to suppress gvh with anti-T as defined in experimental animals was finally introduced to clinical bone marrow transplantation. So far 13 bone marrow transplantations have been performed with anti-T. Although the number of patients is still low, that fact that no fatal gvh occurred and only 2 cases of clinical gvh (grade II) is encouraging.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:
H.J. Kolb, K. Stünkel

Titel des Projekts:
Histocompatibility typing

This project concentrated on tissue typing for bone marrow transplantation into supralethally irradiated dogs. The canine transplantation model is regarded as a preclinical model also what concerns the immunogenetic aspect. Unlike rodents, which represent a degree of compatibility frozen at the level of inbreeding, dogs are outbred with all degrees of compatibility and resemble the human situation, also what regards histocompatibility within a family. Our research dealt with the establishment of the mixed lymphocyte culture test (MLC) in dogs. We then used this test for the definition of lymphocyte defined MLC determinants (DLA-D antigens) in dogs. This was made possible after we had elaborated an approach to type MLC determinants by using homozygous typing cells. When these homozygous typing cells were cocultured with lymphocytes expressing the determinant of the homozygous cell partner in a heterozygous state, stimulation of lymphocytes occurred only in one-way and not both ways because the hyploidentical heterozygous cells tolerate the determinant of the homozygous cell partner. Originally we had this typing assay standardized in the human MLC but it could be applied to the canine situation as well.

MLC testing for bone marrow transplantation is particularly important in dogs, at least to proof DLA compatibility, because serological DLA typing with antibodies is less advanced than its analogue, HLA typing in humans due to the poorer specificity and lack of DLA antibodies for certain spefi-cities.

We then set up a panel of DLA-homozygous typing cells (HTC) for the definition of 11 different specificities (DLA-50 till 60). Compared to human immunogenetics of the analogue HLA-D system it became clear that the species dog possesses a similar polymorphism at the gene loci responsible for the MLC reaction. In addition to the classical MLC typing technique with HTC's we adapted the so-called primed lymphocyte typing (PLT) technique to our canine model. We found that the determinants recognized by the PLT technique are genetically closely related to the classical MLC determinants. The PLT determinants are, however, heterogeneous, i.e. composed of several gene products and in contrast to reports on PLT data in man and pig influenced by a gene dose effect.

Apart from these contributions to formal genetics of DLA-D determinants, MLC testing was used for typing donor-recipients combinations whose degree of incompatibility was compared to the outcome of bone marrow transplantation. It was concluded that DLA-A differences led to failure of engraftment more often than differences in DLA-B and DLA-D ($p < 0.001$). DLA-A differences of the recipient provoked more severe graft-versus-host disease than those of DLA-B and DLA-D ($p < 0.01$). Failure of engraftment or early graft rejection was the rule, when donors differed in DLA-A. Fatal graft-versus-host disease was strongest when recipients differed in DLA-A.

Finally DLA homozygous bone marrow donors and haploidentical DLA heterozygous recipient dogs were identified through one-way MLC typing. This donor-recipient combination was used for investigations on the suppression of graft-versus-host reactions using specific anti-T cell globulin (s. Project No. 3).

In summary histocompatibility typing methods were established in dogs. Original research concerned mixed lymphocyte culture tests with which the first 11 canine lymphocyte determinants could be defined. Exchange of dogs and dog

lymphocytes with other dog laboratories in Ulm and Rijswijk supported by EURATOM lead to a panel of DLA-homozygous-typing cells. Furthermore the DLA-A locus could be identified for contributing to severe graft-versus-host disease. Thus histocompatibility typing revealed findings with direct relevance for the treatment of radiation injury in a preclinical animal model.
During the last year investigations on a better definition of the T antigen in dogs were undertaken. In contrast to man canine thymocytes do not regularly form rosettes with heterologous lymphocytes. Rabbit anti-dog thymocyte globulin contains many antibodies crossreacting with red cells, platelets, B-lymphocytes. Such antibodies can be removed by incubation of the antiserum with great numbers of the appropriate cells. It is however difficult to obtain B-lymphocytes without contaminating T-lymphocytes in sufficient quantities, because B-cells of dogs cannot be grown in cell cultures so far and B-cell tumors are rare. Although spleen cells of newborns puppies appear to absorb more B- than T-lymphocyte activity from rabbit anti-dog thymocyte globulin, it is not easy to formally prove this as long as variable serological anti-T testsystems are not at hand. We recently adapted an immunohistochemical method for the demonstration of T-cells on frozen sections in mice and man.

It is a very sensitive method which even stains the lowly concentrated membrane marker antigens of sectioned cells. It has little unspecific background because the labeling peroxydase-anti-peroxydase complex is conjugated to the marker-specific antibody by a third anti-antibody rather than by chemical bonds. Since T-cells are concentrated to the thymus-dependent interfollicular areas sparing the B-lymphocyte containing follicles, the immunohistochemical staining is a test system of anti-T antibodies also in dogs. After we had an antibody proofed to be T cell specific following various absorptions procedures in this cumbersome indicator system, we developed a more convenient T-cell test.
It consists of binding this antibody by chromium chloride to indicator particles like sheep red cells. We presently test the specifities of anti-T particularly whether it really rosettes only with IgG negative lymphocytes.

Ergebnisse des Projekts Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter:
S. Thierfelder und H. Rodt

Titel des Projekts:
Immunomanipulation of graft-versus-host reactions

Graft-versus-host reactions (gvh) can be live-threatening complications after bone marrow transplantation due to histocompatibility between marrow donor and recipient. Only identical twins or animals of inbred strains are entirely free from these complications. Tissue typing and selection of a histocompatible donor is one approach to reduce the risk of gvh. The probability to find a relatively histocompatible donor is 25% among siblings and very much lower if non-siblings are considered. The genetic polymorphism of the loci coding for tissue antigens leaves no chances that tissue typing will provide histocompatible donors for the majority of patients.

Immunomanipulation of the marrow of histoincompatible donors was therefore investigated in animal models. At least the acute symptoms of gvh are known to be due to thymus-dependent lymphocytes in the transfused marrow of the donor which react with the incompatible tissue antigens of the recipient. We, therefore, concentrated on the question whether these T cells could be eliminated by treating the donor's marrow with specific antibodies and if so whether a T cell deprived marrow could induce the generation of normal T lymphocytes in the recipient which would protect him against third-party antigens.

We, therefore, investigated the effect of heterologous polyclonal - recently also monoclonal - antibodies on gvh in mice. Rabbit anti-mouse-thymocyte globulin was purified from antibodies crossreacting with hemopoietic stem cells. The final anti-T cell globulin proved non-toxic for stem cells

in colony formation and diffusion chamber assays while still lysing thymocytes and T lymphocytes. An incubation of the donor's marrow with anti-T lead to the abolition of gvh in semiallogeneic H-2 incompatible F1 mice. Since recent theories on the organization of immunocompetence doubted that stem cell derived T precursor cells would mature in an H-2 histoincompatible thymus (e.g. of a bone marrow recipient) we investigated chimaeras differing in T cells and thymus by 2 haplotypes. We used thymectomized (C57BL/6 x CBA) F1 hybrids, implanted new-born CBA thymuses under the kidney capsule and transplanted bone marrow of C57BL/6 donors pretreated with anti-T. While control mice with thymus grafts did not form antibodies against sheep red blood cells nor reject third-party skingrafts, all thymus-grafted, bone marrow reconstituted mice developed a normal response of humoral and cellular immunity including also viral immunity. This proofs that under our experimental conditions chimaeras with fully allogeneic H-2 incompatible marrow and thymus can survive with the help of immunomanipulation using anti-T cell globulin.

Recent investigations with monoclonal anti-T raised in our department lead to similar results. This is important because these hybridoma antibodies have the advantage of better standardization which is essential for bone marrow transplantation in patients.

Our studies on the elimination of T cells revealed that rats in contrast to mice express the T antigen already on hemopoietic stem cells, which, of course, precludes the anti-T approach for the suppression of gvh in this species and raised the question whether patients were similar to rats or mice in this respect. Fortunately man could be shown by in vitro techniques to have stem cells without T antigens. This encouraged us to introduce our anti-T approach in clinical bone marrow transplantation also after we had proofed its efficiency in the preclinical canine model (s. project No. 3). So far 13 leukemic patients have been grafted with bone marrow pretreated with anti-T raised in rabbits and

purified in our department from crossreacting antibodies which are toxic for hemopoietic stem cells. Non-fatal gvh occurred in 2 cases. Although the number of cases is still low, reduction of gvh in frequency and severity can be expected from the anti-T approach.

In summary a method to overcome gvh, an important draw-back in bone marrow transplantation, was investigated in a basic animal model and introduced for the treatment of leukemic patients. With the aid of bone marrow transplantation, these patients could profit from a high-dose anti-leukemic whole body irradiation. Thus the present studies contributed to the treatment of a therapeutically applied radiation injury.

Ergebnisse des Projektes Nr. 3

Leiter des Projektes und wissenschaftliche Mitarbeiter:
H.J.Kolb und Bodenberger U.

Titel des Projekts:
Synergistic conditioning agents

Conditioning regimens of bone marrow recipients and bone marrow of the donor in order to improve the survival after high-dose whole body irradiation and bone marrow transplantation was investigated in this project. We used the canine transplantation model since many findings and techniques established in dogs could be directly applied to patients. The studies of this project concentrated on two areas: radiation tolerance and immunosuppression. Conventional treatment of leukemia often fails because chemotherapeutic doses do not eradicate sufficient numbers of leukemic cells. The question was therefore whether the total dose of presently 9 Gy of antileukemic whole body irradiation before bone marrow transplantation could be raised to more cytotoxic levels. Total body irradiation of beagles was studied in detail using two opposing ^{60}Cobalt sources. Hemopoietic toxicity was found to depend less than gastrointestinal toxicity on the dose rate. Therefore higher total doses could be applied with lower dose rates, if bone marrow was transfused thereafter. 250 R, 400 R and 1600 R with dose rates of 55 R/min and 5.5 R/min (air) were apl ied. The higher dose rate suppressed the number of peripheral colony forming cells significantly more. It also induced a more prolonged lympho-, granulo- and thrombocytopenia. 1600 R were lethal inspite of a subsequent bone marrow transfusion. The resulting gastrointestinal toxicity could not be avoided if the dose rate was reduced to 0.5 R/min, at a total dose of 2400 R. A fractionation of the total dose in 600 R and in 600 R together with 1200 R with two days intervals was tolerated if dogs received autologous

- recently also DLA incompatible allogeneic - bone marrow. In a second series of investigations suppression of graft-versus-host reactions (gvh) in DLA incompatible dogs was attempted using absorbed rabbit anti-dog thymocyte globulin. As pointed out in project No. 2 pretreatment of donor marrow with anti-T cell globulin suppressed gvh in mice when parental homozygous donors were used and haploidentical heterozygous H-2 incompatible recipients. Since dogs are random-bred we could not test a genetical donor-recipient situation identical to that used in mice, but similar at least what concerns the major histocompatibility complex. We used dogs as marrow donors, which were homozygous for DLA-DAB determinants and haploidentical marrow recipients which differed from the donor by one DLA-haplotype. Under these conditions little host-versus-graft reactions but clear graft-versus-host reactions had to be expected. Indeed 4 dogs of a group of four in this control series died within 4 weeks after total body irradiation and bone marrow transplantation with the symptoms of acute gvh. In contrast 4 of 8 dogs receiving bone marrow of allogeneic sibling donors which was pretreated by anti-T-cell globulin, survived whole body irradiation and bone marrow transplantation without any symptoms of gvh. These dogs are now 3-4 years after transplantation. They are still chimaeras and in good condition. 2 other dogs of this group suffered from chronic gvh with a survival of a mean of 70 days, 2 dogs died from failure of engraftment. It is not clear whether gvh in the 2 dogs occurred because we used anti-T for pretreatment of the donor marrow at too high a dilution or whether the recipient:donor histoincompatibility was too strong. From experiments in mice we assume that the addition of complement will enhance the effect of anti-T in vitro. Interestingly although the surviving dogs did not show any signs of immunoincompetence, in vitro testing of immune response against sheep red blood cells and haptens remained suppressed for several months after bone marrow transplantation.

In summary our studies demonstrated that tolerance of maximal doses of whole body irradiation can be increased considerably by decreasing the dose rate and by fractionated irradiation.

Furthermore an approach to suppress gvh with antibodies which we established in mice was found to be effective in dogs as well.

It should, however, also be pointed out that incubation of donor marrow with anti-T lead to subacute mortality of fully allogeneic DLA incompatible marrow recipient dogs. It is not clear why immunosuppression was partially successful in one haplotype incompatible chimaeras and failed in dogs differing by two DLA haplotypes from the marrow donor. Rejection of marrow is not infrequent in fully DLA incompatible dogs and occurs seldom in semiallogeneic recipients of DLA homozygous, haploidentical bone marrow. We could not exclude that bone marrow rejection phenomena contributed to the failure of suppression of mortality in our fully DLA incompatible dogs. On the other hand we investigated in mice whether one haplo-type different mice can be distinguished from two H-2 haplotypes differing by the effect to suppress gvh following injection of parental spleen cells. Incubation of donor spleen marrow with monoclonal anti-Th-1 prevented gvh in H-2 incompatible F1 recipients but did not or barely delay gvh in two haplotype-different mice. In the latter group gvh could be suppressed by the addition of complement. From these data it was concluded that there is a competition between antigen-induced proliferation and antibody-derived inhibition of marrow-donor T cells pretreated with anti-T. If there is a two-haplotype difference between donor cells and recipient tissue, the stimulus for antigen-induced proliferation is strong enough to overcome the antibody barrier on the T cells. Only in vitro lysis of T cells with anti-T and complement will suppress gvh. Application of anti-T in strongly incompatible donor-recipient pairs should therefore include both anti-T and complement.

Ergebnisse des Projekts Nr. 4

Leiter des Projekts
Prof. Dr. R. Burkhardt

Titel des Projekts:
Clinical and morphological effects of total and local body irradiation

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General remarks

Radiation damage of the bone marrow is the primary limiting factor of a number of actual therapeutic applications of x-rays as well as the main concern in accidental exposition to nuclear energy. However the strenuos efforts put into clinical observation and experimental work have not yet resolved some of the major problems of its pathogenesis, the control of which is expected to extend the therapeutic range of radiation and to improve the prevention of side effects. In vitro investigations have shown that the undetermined stem cell, the main element of the bone marrow restitution, receives some vital support from the original tissue to maintain its lifelong reproductive capacity (Schofield, Blood Cells 1978). The existence of such an element, never visually identified, is proven indirectly by various experimental techniques (Till and McCulloch, Radiat. Res. 1961, Parmentier et al., Brit.J.Haematol. 1978) and by the fact that haematopoiesis is to be eradicated for lifetime by total body irradiation in a dosage that preserves the stromal tissue of the bone marrow, unless recolonisation is introduced from a shielded marrow region of the same individual or a compatible marrow transplant (Pozzi et al., Curr.Top.Rad. Res. 1973, Croizatet al., Int.J.Radiat.Biol. 1976).

Repeated transplantation of normal undetermined stem cells to several irradiated recipients however results in a linear decrease of the repopulating activity (Siminovitch et al., J.Cell.Comp.Physiol. 1964, Tubiana et al., Path.Biol. 1979), which can be understood as another proof of the reciprocal influence of soil and seed (Patt and Maloney, Exper.Cell Res. 1972, Trentin et al. 1974, Friedenstein et al., Transplantation 1974) and symbolized by the idea of a stromal niche, preserving the optimum conditions for haematopoietic induction (Schofield, Blood Cells 1978). Increasing evidence is accumulated that the lack of a sharp margin of the radiation tolerance of the haematopoietic bone marrow

(Heather et al., Brit.J.Radiol. 1979) is not only due to a puzzle of conditioning cellular, but also stromal factors, contributing to the apparently paradoxical situation of the comparatively low lethal radiation dose, if the whole of the bone marrow is exposed, and a lower rate of repair of the overall haematopoietic capacity of the marrow, when smaller instead of larger compartments of this organ have been irradiated even with smaller doses within the general dose-range of partial radiation (Morardet et al., Biomédicine 1973, Sacks et al., Cancer 1978).

It is evident that more direct information about the stem cell-niche situation as well as direct quantification of the bone marrow activity, generally estimated from scanning techniques, are needed which can be provided only by histology. The use of animal models for this purpose is limited among other things by the vast species-specific differences of the regeneration capacity of the bloodforming tissues and by the fact that therapeutical radiation has to be designed especially for the human diseases that may exert some influence also upon the tissular reaction against radiation (Tubiana et al., Path.Biol. 1979).
The routine bone marrow biopsy of the iliac crest, region of the main haematopoietic activity in the human (Rubin et al., Cancer 1973), therefore offers a unique opportunity for the histological study of these complex phenomena, despite its clinical restrictions. The introduction of improved methods of biopsy and histology have provided us with the technical means. Especially the superior preservation of the stromal tissues and osteological details in semithin preparations of the undecalcified marrow amidst of the osseous framework by the methacrylate technique (Burkhardt, Klin.Wschr. 1966) opens a new way to the morphological study of the marrow tissue by combined light- and electron-microscopy.

Studies and results 1976 - 1980

Two major problems of the histological evaluation of the consequences of radiation in the human had to be faced in the first period of our study: the very slow accumulation of comparable cases due to the conditions of a clinical study, and the lack of experience with the high variability of the stromal reactions of the bone marrow in apparently similar cases as well as the considerable similarity of certain remarkable histological changes under different pathological conditions. Therefore the first aim,

after a preparatory phase, was to classify the basic structural phenomena in patients and bone marrow regions with an exactly defined radiation dosage. The fundamental study of the early and late marrow and bone changes in the pelvic region caused by therapeutic irradiation of genital cancer resulted in the perception of three histologic postirradiation phases: destruction, repair and repopulation. 2 among 15 cases, controlled with 42 biopsies, however showed either delayed or defective repopulation. In the latter case, plasma- and lymphocytic infiltration of the bone marrow preceeded, followed either by marrow aplasia or osteosclerosis. In all cases, osseous remodelling ranged among the most prominent histological changes, accompanied with mesenchymal activation of the peritrabecular tissue, proliferation of arterial capillaries, endothelial cells, histiocytes and osteogenic cells, leaving a disturbed trabecular pattern for many years. It was concluded that radiation caused transient depletion of parenchymal cells, destruction of the sinusoids, thrombotic changes of larger vessels and a longlasting distortion, transferred from one cellular generation to the other, including diminution of the sinusoidal capillaries and inflammatory cellular changes. The type of these changes seemed to influence the reparation.

These changes showed a remarkable similarity with those seen in cases of so-called "nonspecific abacterial myelitis" of rheumatical, immunological or unknown origin, studied in 425 cases , with the marrow aplasia in 406 cases of aplastic anaemia and with the bone marrow changes in 112 cases with haematological neoplasias after polychemotherpy, investigated before and during treatment . In most of these cases the primary event appeared to be necrosis of the haematopoietic cells, followed by a cellular reaction of the type of delayed hypersensitivity and necroses of the smaller vessels.

To evaluate the role of these vascular changes in the atrophic and fibrotic bone marrow distortion, a great number of structural variables have been controlled together with the number of the vascular cross-sections in the bioptical slides of 40 cases of each of 10 disease groups . Whereas a regular proportion of arterial: venous capillaries was maintained in the normal, the atrophical conditions of the bone marrow and bone were accompanied with diminution of the capillaries, the one more pronounced on the venous, the other on the arterial side. Overproduction of erythropoiesis

showed massive increase of both types of capillaries, proliferation of the granulocytic series mostly of arterial capillaries. Antagonistic relations were found for the proliferative haematopoiesis on one hand and increased osseous remodelling activity on the other, each one occupying a significant part of the capillary supply. The before mentioned type of mesenchymal activation was found in similar shape in different groups, suggesting the special affinity of the peritrabecular marrow tissues to angiogenetic and osteoblastic activities, finally occupying the whole marrow space. There were two conditions excluding a niche-function: the cellular and capillary depletion of the fatty and the granulomatous organization of the fibrous marrow. Evidently the capillary structure imprinted the arrangement of the haematopoietic and osseous tissues in all groups.

The parallel observation of the prompt restitution of the total edematous marrow depletion in 5 cases of anorexia nervosa , the morphological identity of the non-haematopoietic fatty marrow in aplastic anaemia with the potentially haematopoietic fatty tissue in the central parts of the long bones, and the restitution or progress of bone marrow fibrosis under certain conditions, shows that additional factors are responsible for the different outcome of similar structural distortions. Inflammatory, especially lymphocytic marrow infiltration, seems to mark primarily the progressive atrophic and fibrotic stages.

From these observations the following supplementum is proposed to the niche-theory: some exclusivity exists between the different bone marrow performances to shelter or even to procreate haematopoiesis or to react as a matrix either for lipid storage and exchange, or mechanical repair including osteogenesis; the former representing the most distinguished and vulnerable function. The experience of similar structural changes of unknown and known exogenous etiology, including the case controls in 303 patients with haematological neoplasias , reversible under certain conditions, supports the hypothesis of a universal stromal bone marrow matrix instead of a definite number of niches, imprinted with a programme of which single performances can be selected or destroyed by radiation as well as other stimuli, normally balanced by a feedback mechanism and dependant on a suitable capillary supply. The common regression of the haematopoietic

and osseous tissues in the flat bones due to immobilization, which is more prominent in the younger, is in accord with this hypothesis (R.Bartl: manuscript in preparation).

In the scope of this assumption two results are to be mentioned especially: the pertinent diminution of the bone marrow sinusoids in the irradiated cancer patient , and the same event together with a reduction of the number of the megakaryocytes in a long term survey of patients with polycythaemia vera, treated with radiophosphorus, or untreated . In the observed 59 cases, studied with 151 bone marrow biopsies in the course of 2-15 years, the development of myelofibrosis did not correlate with the therapy but with the number of megakaryocytes. The same was stated in a survey of 415 cases of CML . As has been confirmed by others, marrow fibrosis is not to be reckoned among the direct consequences of therapeutic radiation. Capillary alterations however are to be regarded as candidates of a further survey of the structural factors possibly contributing to the irreversibility of the immediate cellular depletion of the bone marrow caused by tumoricidal x-ray dosage.

Work in progress

To identify the above named basic principles of bone marrow repair and reactive distortion, two series of investigations are in progress: the computerized histological analysis of 613 cases with nonspecific abacterial myelitis, and of 542 cases with haematological neoplasias, including the lymphoma group and Hodgkin's disease, treated with different irradiation schemes or polychemotherapy. The aim is to compare the nonspecific changes of the bone marrow matrix, haematopoiesis and osteogenesis of different etiology, to control the matrix hypothesis and to collect indirect information of the influences on and the interrelationship of the different forms of aplasia, fibrosis and inflammation, as the common elements of radiation damage.

Data of the fatty tissue and other stromal components of the bone marrow have been collected in 20 cases of each of the following groups: 3 normal groups of different ages; aplastic anaemia; and marrow aplasia due to radiation, osteoporosis, malnutrition, rheumatoid arthritis, acute leukemia, plasmacytoma, metastatic carcinoma, and Paget's disease. The analysis will be completed in 1981.

In coordination with the transplantation programme, the bioptical results before and after treatment are collected for the retrospective evaluation concerning indication, prognosis and the matrix hypothesis.

Summary

Improved methods of biopsy and histology have been used to gather morphological evidence about the pathogenesis and consequences of the therapeutic radiation of the human bone marrow. The comparison with other non-neoplastic bone marrow distortions of known and unknown etiology suggests the substitution of the niche-theory of the regeneration of the haematopoiesis by a new matrix hypothesis, postulating the general programmatic ability of the marrow stroma to introduce exclusively haematopoiesis, osteogenesis, immunopoiesis, and fibrosis, assisted by an adequate microcirculation, to be stimulated or suppressed by radiation as well as various other causes. It is intended to control this concept in a larger series of patients with different forms of radiation and chemotherapy and especially to evaluate the general bone marrow consequences of different volumes and dosages of radiation by morphometrical means. From the results of this project a more exact base for the application of functional and experimental data together with direct insights in the field of therapeutic application of, and protection against radiation in the human is to be expected.

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Contractor:	University of Naples
No. of contract:	159-76-7-BIOI
Principal investigator:	Cesare Peschle M.D.
Subject of the contract:	Radiation and marrow aplasia: stem cell kinetics after prolonged or long-past radiation.

Abbreviations: Spleen, myeloid-macrophage or erythroid colony-forming unit: CFU-S, CFU-C, CFU-E. Erythroid burst-forming unit: BFU-E. Erythropoietin: Ep. Granulo-monocytic colony-stimulating factor: GM-CSF. Burst-enhancing factor(s): BEF.

1) Mechanisms regulating kinetics of early hemopoietic precursors under normal conditions and following radiation.

A) A series of studies has been performed on the influence of Ep and BEF at the level of erythroid precursors, both under normal conditions and after exposure to radiation or treatment with radiomimetic agents.

These studies clearly indicate that Ep largely or exclusively controls: (a) the kinetics of the CFU-E pool (pool amplification, and also in part the percentage of precursors in DNA synthesis); (b) the survival of CFU-E (in the absence of Ep, CFU-E do not survive in vitro, and also presumably in vivo); (c) the final differentiation step from CFU-E to erythroblasts, and hence the erythropoietic rate.

On the other hand, BEF largely controls proliferation, survival and initial differentiation of "primitive" BFU-E. Thus, animals exposed to radiation (and then injected with normal marrow) or injected with radiomimetic agents (and then showing endogenous marrow repopulation), show enhanced cycling of BFU-E (unaffected by transfusion) and a wave of BFU-E—FU-E differentiation (dampened by transfusion). This is associated and ost likely mediated via enhanced BEF levels in serum (and hence in the

hemopoietic microenvironment).

Ep may also exert some action on primitive BFU-E proliferation and differentiation.Thus, transfused mice show an early decline of BFU-E number and cycling, which is promptly followed by a rebound of both parameters towards normality. This biphasic phenomenon is possibly mediated by an early sensitivity of BFU-E to the transfusion-induced drop of Ep activity. This may be followed by a compensatory rise of BEF activity in serum, which induces both parameters to rebound up to normality, thereby masking the early Ep sensitivity.

B) Studies on the PGE-cAMP influences on erythroid precursors showed that PGE's dampen or enhance BFU-E growth, respectively in mice or humans. The action on human precursors is abolished by prior removal of adherent cells.

C) Three classes of erythroid precursors have been identified in human fetal liver (and confirmed to exist in human marrow): "primitive" BFU-E, "mature" BFU-E and CFU-E. Both in fetal liver and adult marrow BEF is required for expression of BFU-E but not (unless to a minor extent) of CFU-E. The Ep sensitivity of fetal precursors is progressively enhanced along their differentiation pathway, and is more marked than for corresponding adult progenitors. BEF is released in marrow but not in fetal liver by adherent cells.

2) Significance of the above studies and their projection on future investigations.

It is suggested that the above studies, substantially in line with the originally-proposed research project, have contributed to extend our understanding of mechanisms (BEF, Ep, PGE, etc) regulating the kinetics of erythroid precursors, both under normal conditions and following radiation. More important, they seem now to undergo a crucial development, in that BEF apparently plays a key role in modulating the kinetics of erythroid precursors, and more particularly mediates, at least in part, thei post-radiation recovery. Indeed, preliminary results indicate that: 1) BE

is elevated in murine serum after radiation, 2) injection of crude BEF from syngeneic normal mice into recipient, mildly-irradiated animals induces an enhanced recovery of erythro- and hemopoiesis in the latter group. Furthermore, studies on the relationship between BEF, GM-CSF and a factor modulating CFU-S cycling shall represent a further, crucial development and extension of investigations performed so far.

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B) Journal Publications

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Associato della Commissione: Comitato Nazionale per l'Energia Nucleare
No. del Contratto: 173-76-1-BIOI
Capo del Gruppo di Ricerca: Prof. G. Doria
Tema generale del Contratto: Radiation sensitivity and recovery of the immune system.
Collaboratori scientifici: Dr. L. Adorini, G. Agarossi, G. Gorini

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The aim of the studies carried out during the period 1976-80 was to investigate the effects of whole-body irradiation on the immune system at the humoral and cellular level. The main findings were the following: 1) Enhancement of antibody affinity in irradiated mice. 2) Existence of two subpopulations of helper T cells with different radiosensitivity. 3) Recovery profile of T and B cell populations in irradiated mice.

1) Enhancement of antibody affinity. This antibody property reflects the binding energy of the antibody sites for antigen and affects the stability of the antigen-antibody complexes. Thus, antibody affinity influences the antibody's ability to neutralize viruses and toxins. Since affinity is important to counteract infections in irradiated animals, studies were under taken to examine affinity during the antibody response in mice exposed to a sublethal dose of X-rays. Affinity was found up to 20 times greater in irradiated than in control mice when antigen was injected 1-5 days before or 2 hr-8 weeks after a sublethal dose of X-rays ranging from 25 R to 450 R. The observed enhancement of antibody affinity may result from a relative lack of suppressor T cells and play an important role in radiation-induced immunodeficiencies as it could overcome quantitative defects of antibody-producing cell populations and assure the survival of the animal recovering from radiation damage.

2) Radiosensitivity of helper T cells. Helper T cells partecipate in the processes of antibody response against all microorganisms that can invade the organism when immunological defence mechanisms have been impaired by irradiation. Since the radiosensitivity of helper T cells was a controversial issue owing to complications met when the evaluation was performed in vivo,

the helper activity of T cells, primed and irradiated in vivo, was tested in vitro to establish a radiation dose-response relationship. Spleen cells from mice carrier-primed 4 days before exposure to 50-2000 R were tested for their ability to help syngeneic normal spleen cells to mount an in vitro anti-hapten antibody response after stimulation with the hapten-carrier conjugate. The curve describing the remaining helper activity as a function of the radiation dose showed the presence of two components, one more radiosensitive than the other one, suggesting the existence of two helper T cell subpopulations. It was also found that the radiosensitive subpopulation increases with the time interval between carrier-priming and irradiation, and can be separated from the radioresistant subpopulation by nylon wool filtration. The type of functional alteration resulting from depletion of the radiosensitive subpopulation of helper T cells remains to be determined. 3) Recovery of T and B cell populations. During 1980, the same method used for in vitro evaluation of the helper activity of differentiated T cells from mice carrier-primed before irradiation was applied to helper cells from mice primed at different times after different radiation doses to assess the recovery of precursor T cells. The immune reactivity of B cells from the same mice was also estimated in vitro by use of a T-independent antigen. The early recovery at 1-2 weeks after 50-200 R was found to be greater for T than for B cells. From experiments performed with pooled spleen cells, both T and B cell populations appear to recover completely at 3 months after 300 R. However, when the same methods were applied to spleen cells from individual mice immune defects could still be detected at 3 months after 200 R. Yet, recovery at 6 months was complete even after 400 R, regardless of whether spleen cells from pooled or individual mice were used. These findings altogether suggest that radiation-induced perturbations of immune homeostasis can eventually be overcome, spontaneously. However, since recovery is fully attained after an extensive period of time, the irradiated individual is likely to suffer the late consequences of a long-lasting unbalance of the immune surveillance mechanisms.

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Contractant van de Commissie :	Vrije Universiteit Brussel
Nummer van het contract :	263-78-1 BIO B
Hoofd van het researchteam :	R. HAMERS
Algemeen onderwerp van het contract :	Biological Effects of Irradiation

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Algemene omschrijving van de uitgevoerde werkzaamheden met de nadruk op de betekenis van dit onderzoek voor het stralenbeschermingsprogramma van de Gemeenschap.

The first investigation concerning radiation and immunity were performed prior to development of cellular immunology. These early data only described the final effect of irradiation on a particular immune reaction. Today we know that an immune response is the result of a complex series of interactions between different lymphoid cell types, and more attention is paid to the effect of radiation on these individual cell populations. Much progress has been made in the field of cellular immunology because techniques became available for the in vitro induction of an immune response as well as for the separation of functional lymphocyte subpopulations. The isolation of different lymphocyte populations and their functional characterization has led to a better understanding of the immune network. It is quite clear now that besides positive cellular cooperation (help), there also exist negative interactions (suppression). Moreover, suppression seems to be dominant. Suppressor mechanisms not only protect the individual from developing to excessive immune reactions

upon infection with a foreign antigen, but also from reacting against self-determinants (such as is the case in auto-immune disease). Since an intact control mechanism seems to be of utmost importance for accurate functioning of the immune system, we decided to concentrate our research on the effect of irradiation on the regulatory compartment. As an experimental system we found the rabbit more suitable than the inbred mouse because of the presence of well-defined genetic markers on its immunoglobulins (allotypes) as well as markers belonging to the quasi-silent gene products. Moreover, working with a non-inbred population is more relevant to the situation one has to face when investigating human material.

Resultaten van het project No. 1

Hoofd van het team en wetenschappelijke medewerkers : R. HAMERS

M. Vaeck, W. De Smet, W. van der Loo, P. De Baetselier, C. Casterman.

Titel van het project :

Effect of irradiation on the expression of immunoglobulin genes

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Beschrijving van de resultaten

Some preliminary experiments were undertaken to evaluate the effect of whole body Xray irradiation (2500r) on the rabbit immune system. We noticed a decline in the proportion of splenic B to T cells during the first weeks after irradiation, as well as an altered mitogen reactivity of the spleen lymphocytes. Most interestingly when rabbits were immunized shortly after irradiation with the antigen sheep red blood cells (SRBC), they showed a significantly enhanced primary antibody response against this antigen. This observation would be in accordance with the hypothesis that irradiation primarily affects immune regulation. However, other mechanisms such as gut damage or altered cell homing could not be completely ruled out as the main cause of this enhancement. However we felt that, in order to elucidate the exact nature of this effect, we needed to use more sophisticated techniques such as the in vitro culture of lymphoid cells.

This would enable us to manipulate more easily the different cellular components of the immune system and to obtain direct information on the radiosensitivity of the various cell types involved in immune reactions.

Extensive studies, conducted in our laboratory, using a cell culture system for the in vitro induction of an immune response with rabbit peripheral blood lymphocytes (PBL), revealed that two different suppressor cell types regulate antibody production: one radiosensitive (25-50 r), the other radioresistant (at least up to 2500 r).

Radioresistant suppressor cells.

PBL from SRBCprimed rabbits could be restimulated in vitro to generate a secondary antibody response against this antigen. Both IgM and IgG secretion (measured by the plaque forming cell test) were significantly enhanced by fractionating the PBL on insolubilized histamine. Readdition of the histamine binding cells (H^+ cells) to cultures of histamine non-adherent cells (H^- cells) resulted in strong suppression. The H^+ suppressor cell was sensitive to treatment with antiT serum and complement but was insensitive to in vitro Xray irradiation with doses up to 2500 r. Hence the H^+ suppressor cell is a radioresistant T cell.
Apparently the finding of radioresistance is incompatible with the general hypothesis that suppressor cells are relatively radiosensitive. However, from the study of primary in vitro responses, we have obtained evidence that the H^+ suppressor cell is antigen-induced. Thus the possibility exists that in vivo priming has induced the differentiation of radiosensitive suppressor precursor cells into radioresistant effector cells.

Radiosensitive suppressor cells.

When PBL from immunized rabbits were depleted of H^+ cells and irradiated with low doses of Xrays (2500 r) the secondary antibody response was consistently enhanced. Such a supplementary enhancement in the absence of H^+ suppressor cells, induced by low dose irradiation, is indicative of the existence of a radiosensitive histamine non-binding (H) regulatory cell in PBL of immunized rabbits.

We also established the experimental conditions for the in vitro induction of primary antibody responses with rabbit PBL. In contrast to the secondary response, primary antibody production in vitro was not altered by chromatography of the cells on insoluble histamine. However, low dose irradiation of unfractionated or H^- PBL resulted in significantly higher responses. Thus although the radioresistant H^+ suppressor cell is only present in PBL from immunized animals, the radiosensitive H^- regulator cell seems to be independent of in vivo immunization. Whether the latter one respresents an earlier maturation stage of the H^+ suppressor cell or whether it is a completely different regulatory cell type remains to be determined.

The results obtained from these in vitro experiments are in complete agreement with our initial data concerning enhancement induced by in vivo low dose irradiation. Moreover the data clearly indicate that such enhancement is due to a direct effect on a lymphoid cell population (probably a radiosensitive suppressor cell) and rule out the possibility of an indirect effect such as gut damage or altered cell homing as the main cause of radiation induced enhancement.

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Congress of Immunology, Paris 1980. Congress Proceedings, Abstract nr 4.2.24. 4.

Contractant de la Commission : Université Libre de Bruxelles

N° du contrat : 230-76-1 BIO B
Chef du groupe de recherche : Jacques E. DUMONT

Thème général du contrat : Effect of irradiation on leucocyte, hematopoiesis and thyroid - Definition of new biochemical markers

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Titre du projet n° 1 : Biochemical Markers of Leucocyte Function

Chef du projet et collaborateurs scientifiques : E. SCHELL-FREDERICK

A. Biochemical Markers

Phagocytosis by polymorphonuclear leucocytes represents the major defense of the organism against bacterial invasion. Our work concerns the biochemical mechanisms of phagocytosis with particular emphasis on the control elements involved. During the period of the contract, we have concentrated our attention on two possible control elements, calcium and prostaglandins.

We have shown that phagocytosis is accompanied by an increased release of ^{45}Ca from prelabelled leucocytes. The increase is not dependent on phagosome formation, parallels the release of granular enzymes into the medium and is markedly inhibited when the cells are labelled at 0°C. These results have led to the hypothesis that calcium is actively stored in leucocyte granules and discharged into the medium during phagocytosis together with granular enzymes. This increased efflux would represent the final step of stimulus-secretion coupling in the leucocyte. Studies carried out in 1980 using inhibitors of granular enzyme discharge (low temperature 4-acetamido-4'-isothiocyanostilbene-2,2'-disulfonic acid, p-bromphenacylbromide, nordihydroguaiaretic acid) have shown parallel inhibition of ^{45}calcium release, thus bringing added support to this hypothesis. Direct measurement of the capacity of isolated leucocyte granules for active calcium uptake and to detect the possible presence of Ca-ATPase activity have been hampered by technical problems. Satisfactorily pure preparations of granules have been prepared on Percoll gradients, but the addition of ATP to such granules results in a dense precipitate, thus preventing studies of active calcium transport.

Evaluation of primary prostaglandin formation in guinea pig neutrophils has shown no consistent effect of phagocytic particles or cytochalasin E on

PGE_2 or $PGF_{2\alpha}$ production. In an effort to determine whether production and release of primary prostaglandins occurs in bacteria and may be important in the pathogenesis of infection, we measured such production in common saprophytic and pathogenic bacteria. No significant PGE_2 or $PGF_{2\alpha}$ formation was detected. PGE_2-like substances are produced in some strains of Propionibacterium acnes. The possibility that bacteria may produce other prostaglandins (for example, lipoxygenase products) has yet to be studied.

B. Irradiation

Contrary to the results of previous in vivo animal work, we have failed to detect changes in biochemical markers (random mobility, chemotaxis and iodination) of leucocyte function in patients undergoing irradiation for pulmonary neoplasm. This suggests that high doses of irradiation administered to regions of active bone marrow may be necessary to alter leucocyte function and that the main effects of irradiation on leucocytes bear on their formation rather than on their function.

As infections and abcesses are important consequences of irradiation, methods to localize silent abcesses were investigated. Labelling of leucocytes with ^{111}Indium for use in the detection and localization of infection has been initiated in 1980. Making use of our experience with ^{99}Tc-sulfur colloid we have been able to proceed rapidly to clinical utilization. To date, we have performed 90 diagnostic prodecures. We have had two false positives and no verified false negatives. The radiation load is minimal. With this technique primary and metastatic tumors are not labelled. Heterologous donor leucocytes may be used in neutropenic patients. This useful test for the detection of occult inflammation in irradiated patients is available.

In a further effort to identify biochemical markers of the effects of irradiation, we have initiated studies of PGE_2, $PGF_{2\alpha}$ and cyclic GMP formation in dog thyroid slices and renal medulla. Free radicals are known to be generated by irradiation and these activated molecules have also been implicated in the formation of prostaglandins and cGMP. These appears to be no consistent effect of irradiation on these parameters in resting or carbamylcholine-stimulated thyroid slices.

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Titre du projet n° 2 : Effect of irradiation on the regulation of erythropoiesis and erythropoietin

Chef du projet et collaborateurs scientifiques : J.P. NAETS

These last 4 years, we have studied the catabolism of erythropoietin in marrow aplasia either secondary to chemotherapy or X Ray irradiation. We have observed that the catabolism of the hormone is not affected by drug aplasia but reduced after irradiation.

On the other hand, we have assessed the action of glucagon in erythropoiesis and have pointed out the inhibitory effect of this hormone.

1. Compared effects of irradiation and cyclophosphamide (CTX) induced erythroid aplasia on the catabolism of exogenous erythropoietin.

It has been shown that the plasma clearance rate or erythropoietin after hypoxic stimulation is reduced in irradiated rats. This effect has been atributed to medullary aplasia following irradiation, and a relationship between cellularity of the erythroid marrow and erythropoietin utilisation was inferred (Stohlman and Brecher, 1959), on the other hand we have shown that utilization of endogenous erythropoietin was not increased in the dog with hyperplastic marrow (Naets and Wittek, 1965) nor reduced in the rat with experimentally induced marrow aplasia (Naets and Wittek, 1969). As endogenous erythropoietin could be produced for a longer time after arrest of hypoxic stimulation in the irradiated groups, we studied the clearance rats of exogenous instead of endogenous erythropoietin in irradiated and CTX treated rats.

Haematological values were similar in irradiated and CTX treated rats. 42 h. after irradiation (400 r) of CTX administration 50 mg/kg body weight) the marrow was deeply aplastic in both groups. Whereas the femur of a normal male Wistar rat contained 29 x 10^6 normoblasts, only 1.64 and 1.36 x 10^6 normoblasts remained respectively after irradiation or CTX administration. Plasma with high erythropoietin activity obtained from rats of the same strain

submitted to hypoxia (17 h., 270 mm Hg) was injected by a tail vein. The erythropoietin titer was measured on pooled plasma of groups of 4 rats killed 0 (10 min), 1, 2, 4 and 6 h. after the injection. After irradiation the $\frac{I}{2}$ was increased from 1.5 h. in controls to 2.3 h. In spite of similar aplasia, the $\frac{I}{2}$ observed after CTX administration was identical to the $\frac{I}{2}$ of controls.

These results suggest that the action of X rays on the catabolism of erythropoietin is independant of erythroid aplasia and could be related to an extramedullary effect of irradiation.

2. Inhibitory effect of glucagon on erythropoiesis

Anemia of obscure origin is a common feature in glucagon secreting tumors. Regression of the anemia after surgical removal has been reported. This association between hyperglucagonemie and anemia might be fortuitous. We have therefore studied the effect of glucagon on erythropoiesis in mice and rats and demonstrated an inhibitory effect of the hormone.

Long-acting glucagon protamine zinc (NOVO) was injected subcutaneously twice daily at 9 a.m. and 4 p.m. After administration of 2 x 200 µg/day for 10 days, the erythropoiesis of male rats was markedly depressed. Total normoblasts counts per femur, reticulocytes, and ^{59}Fe uptake into red cells fell respectively to 35 %, 50 % and 17 % of control values. The same results were observed with male and female mice injected twice daily with 50 µg of glucagon. The erythropoietic response of mice to hypoxia was also inhibited. After 12 days of hypoxia, (320 mm Hg, 16 h./day), the red cell mass increased in controls from 3.12 to 6.31 and only to 5.19 ml/100 g body weight in the glucagon-treated group ($p < 0.02$). Response of polycythemic mice to exogenous erythropoietin (ESF) was reduced after glucagon injection, and the inhibition was proportional to the logarithm of the dose. The lowest active dose (3 µg) reduced the ^{59}Fe uptake to 77 % of controls, whereas 50 µg administration reduced it to 43 %. This inhibitory effect decreased gradually as a function of the time interval between glucagon injections and ESF administration. Production of ESF seemed unaffected by glucagon administration, since after hypoxia (4 h., 300 mm Hg), the ESF titer of rats and mice was similar in controls and glucagon-treated groups. As the inhibitory effect of glucagon was only elicited if administrated close to the ESF injection, and since no effect on ESF production could be demonstrated, it was inferred that the

hormone acts mainly at the level of erythroid stem cell differentiation. It was suggested that hyperglucagonemia was responsible for the anemia of glucagonomas and might be implicated in the anemia of other clinical conditions with hyperglucagonemia. This could be the case of anemia in hepatic cirrhosis, chronic inflammatory syndrome and renal failure. On the other hand, glucagon acts in most cells and presumably on bone marrow by generating intracellular cyclic AMP. Our results therefore suggest that systematic pharmacological manipulation of cyclic AMP levels in erythropoietic cells in the bone marrow will provide new therapeutic tools in the control of hematopoiesis.

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Titre du projet 3: Thyroid as a target and model of irradiation injury

Chef du projet : J.E. DUMONT

Surveys of patients whose neck has been irradiated accidentally or for a variety of diseases 10 to 20 years before, reveal a large and progressively increasing incidence of thyroid nodules and cancers. Such findings raise the question of the possible consequences of the numerous radioiodine uptake tests carried out for the diagnosis of thyroid diseases and of possible accidental release of radioiodine release in nuclear accidents. Until now, in animals, the best short term in vivo index of radiation damage to the thyroid is the release of thyroglobulin. With Van Herle (San Diego, USA) we have set up in the laboratory a very sensitive assay for human thyroglobulin. 10 patients who had been sent for a thyroid scan and PBI^{131} determination were investigated before and after administration of a dose of 50 µCi of I^{131}. Basal serum thyroglobulin levels varied much from one patient to another (1 to 80 ng/ml; mean 32,7 ± 29,5 S.D.) but was consistent for any individual. 24 h. after I^{131} administration, serum thyroglobulin levels were unchanged (33,0 ng/ml). Thus within the limits of validity of this method no thyroid damage has been demonstrated for this radiation level. In order to check a wider range of dose, a dog serum thyroglobulin radioimmunoassay has now been set up in the laboratory.

To investigate such problems as the lifespan of the differentiated thyroid cell as well as the biochemical mechanisms accounting for the radiosensitivity of this cell, the establishment of cultures of growing differentiated follicular cells is necessary. Preliminary experiments suggest that dog thyroid cells in primary culture might provide such a system. We have succeeded in developing a system, which for the first time satisfactorily meets these criteria. Our primary dog thyroid cell cultures retain some elements of differentiation (iodide trapping, cyclic AMP response to TSH, thyroglobulin synthesis); their slow growth is enhanced by TSH by a cyclic AMP mediated action, their differentiation (as evidenced by thyroglobulin synthesis) can be modulated by various agents such as butyrate. The fact that growth in this system is activated by cyclic AMP shows that this intracellular signal is not, contrary to a widely held concept, a universal negative signal for growth.

The application to man of experimental work carried out in animals requires satisfactory evidence that both systems are comparable. We have therefore defined various aspects of metabolism in the human thyroid and their regulatory network. This work now provides the background for the study of biochemical markers of differentiation in spontaneous and irradiation induced thyroid nodules.

Various aspects of radiobiology have been investigated using the thyroid as a model. With Davies (Omaha, USA), contrary to previous evidence, we have been unable to observe consistent effects of radiation on polysome function or binding to ergastoplasmic membranes. A cyclic GMP has been shown to monitor radicals in various tissues including the thyroid, we have studied the effect of radiation on cyclic GMP levels as a biochemical marker. Cyclic GMP in plasma and urines of rats irradiated in vivo (with Gerber, Mol) was not consistently increased. Very variable results have been obtained in acutely irradiated dog thyroid cells. It is therefore difficult to accept the hypothesis that cyclic GMP is a marker of free radicals in tissues.

A synthesis of the work carried out on irradiation of the thyroid and its consequences has been presented with the Dublin's group in a monograph published by the EEC. It started a useful European collaboration. This work has lead to propose a directive to the Commission for health protective measures to be adopted for the prevention of harmful consequences of nuclear accidents.

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Contractant de la Commisson : UNIVERSITE CATHOLIQUE DE LOUVAIN

N° du Contrat : 250-77-1 BIO B

Chef du groupe de recherche : H. BAZIN

Thème général du contrat : Consequences of irradiation on the local intestinal immunological system. Prevention and treatment of their effects.

Titre du Projet : Consequences of irradiation on the immunological system

Chef du Projet : H. BAZIN

Irradiation of the total body or localized on the intestine can lead to fluid and electrolyte depletion. In the range of infra or midlethal doses of irradiation, septicemiae can also occur. The features of bacterial infections due to ionizing radiations seem relatively peculiar to them. Their most important characteristics are :

- The bacteriae implicated in the invasion are generally considered as poorly agressive or not at all pathogenic for the host. They generally come from the normal enteric flora.
- The occurrence of bactermiae appears during the second week after irradiation.

The pathogenesis of microbial infections after radiation injury is still poorly understood. As a matter of fact, the normal defences against microorganisms are numerous and generally divided into natural or acquired resistances. Both are mediated by different mechanisms, either mechanical (mucociliary system...) or chemical (lysozyme - acidic pH of secretions...), at the exterior of the organism. Antibodies play a major role by covering up the receptors of the bacteriae or of the epithelial cells at the surface of the mucosae or of the skin. Many systemic protective mechanisms also exist, such as the C reactive protein, acute inflammatory response, "natural" antibodies and many cells such as granulocytes, macrophages,... which act with or without antibodies or complement. Another factor of complexity is the great number of microorganisms which can be involved in septicemiae, each bacteria having a distinct type of pathogenecity due to its own characteristics. The effects of ionizing radiations on such a diversity are difficult to study. Moreover, the success obtained by chemotherapy has been considered so important in the fight against microorganisms that it has, these last years, dissuaded further efforts in this field.

In fact, septicemiae are still very frequent and still kill a high percentage of patients, mainly those suffering from immunodepressions such as those due to irradiation. The deficiencies of the antimicrobial defence mechanisms which appear after irradiation have to be studied in terms of modern immunology.

The first consequences of irradiation for the intestinal tract, at doses up to midlethal, seem to be : failure of the biological barrier constituted by the intestinal epithelium; depression of the local and general immune responsiveness and changes of the intestinal flora with an increase of pathogens or conditionally pathogenic microorganisms by disturbance of normal bacterial antagonism. The barrier function of the intestinal mucosa is certainly one of its most fundamental properties. The simple columnar epithelium that lines the gastrointestinal tract must prevent the entry of dangerous antigenic substances and microorganisms into the organism. Irradiation can result in a marked reduction in number and size of the epithelial cells. These abnormalities reached a maximum by the third day after irradiation. However, they are not associated with gross drastic discontinuities in the epithelial lining. Mice irradiated with a coesium source at doses from 5 Gy to 40 Gy from the day of irradiation up to day nine after it, were used to determine to what extent the impermeability of the gut barrier was impaired. Iodinated human serum albumin was administered by gastric intubation after a fasting period of 12 hours. We observed no significant increase of the entry of labelled molecules for the animals given a gamma ray dose below or equal to 1,000 rads from day 0 to day 9, by comparison with the control mice. However, a slight increase of absorption was observed for doses equal or higher than 20 Gy. These results showed that high irradiation doses can increase the absorption of antigenic molecules. We have also analyzed the pathways through which molecules or small particles can escape the blood vessels and pass through the intestinal epithelium into the gut lumen. Three markers of different molecular weight were employed : horse radish peroxidase (Mw : 40,000), horse ferritin (Mw : 600,000) and thorothrast (size from 50 to 1,000 A°). From one to five days after a 9.5 Gy X-irradiation, mice were inoculated with one of the markers. In normal mice, ferritin and thorothrast molecules were found localized in the blood capillaries. In irradiated mice, these molecules were identifiable in the capillaries and in the perivascular spaces. The abnormal escape of these molecules was due to pinocytosis but also to abnormal spaces between the endothelial cells. But in no place, as in the former experiments, were the terminal bars between epithelial cells seen broken and no direct and complete passage was observed. In conclusion, in sublethally irradiated animals,

there is no clear rupture of the intestinal epithelium. The passage of soluble or particulate substances or microorganisms cannot be a direct diffusion mechanism across the intestinal mucosa, except probably in rare cases.

The second approach of the pathogenesis of post-irradiation infections was the general and intestinal immunity. The host defence mechanisms against infections are multiple. They could be divided into specific and non specific factors. We have begun to analyse the case of a non-pathogenic microorganism for the rat : Yersinia enterocolitica, which is found in sporadic human infections all over the world. Rare Y. enterocolitica septicimiae can occur in humans but they have always been reported in immunodeficient patients. The strain of Y. enterocolitica that has been used in our studies was found non-pathogenic for all tested rat strains up to the dose of 10^9 microorganisms. Higher doses were lethal for rats, even when the 10^8 organisms were killed before inoculation. But doses of 10^8 microorganisms were also found non-pathogenic for Nude (athymic) rats, rats deprived of B lymphocytes (by mu neonatal suppression) or both. Doses of Y. enterocolitica up to 10^8 organsims were unable to kill rats when injected 24 hours after an irradiation exposure of 4 to 7 Gy. On the contrary, rats given 10^8 Y. enterocolotica five or ten days after a 5.7 Gy gamma ray exposure were killed by septicemiae. These results demonstrated that immunity mediated by T ot B lymphocytes is not always implicated in post-irradiation infections. Factors such as macrophages, granulocytes, eosinophils, are presently under study.

However, in cases of pathogenic microorganisms, immune responses may have an important role in the defence of the host. We have chosen as model the IgE immune response which is known to be completely T-dependent and we have studied its radiation induced enhancement (RIE). We have demonstrated a) that the RIE phenomenom can be observed in some rat strains but not in others; b)that it is dominant in F1 hybrids between two strains, one being RIE positive and the other negative; c) that the genome coding for the kappa and the heavy Ig chains cannot transform an RIE negative strain into a positive one; d) that the transfer of the major histocompatibility complex of a positive strain to a negative one does not change its RIE status. These results must be interpreted in terms of production of antibody after irradiation and can explain differences in susceptibility to microorganisms between immunodeficient patients.

Further studies will be necessary to properly consider the role of their antibody production in the pathogenesis of infection after irradiation.

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Contractor: Lab. di Radiobiologia, Istituto di Radiologia, Università di Firenze

Contract No.: 257-77-9 BIOI

Head of research team: A. Becciolini

Title of project: EFFECT OF MULTIPLE DAILY FRACTIONATION OF THE DOSE IN THE SMALL INTESTINE OF RATS.

Head of project and coworkers: A. Becciolini, M. Balzi, A. Benucci, S. Cinotti, D. Cremonini, D. Fabbrica, F. Franciolini, V. Giaché, A. Lanini, A. Nardino

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The mechanisms producing radiation damages and cellular repair processes are known enough in cellular systems "in vitro", but they are not yet known in tissues because of the different complexity of the system.

In order to consider these parameters the small intestine was studied as a model for tissues with a high proliferative activity. The microscopic anatomy of this tissue allows to distinguish the proliferative compartment from the differentiated one, so that the small intestine appears to be a good test to follow the changes in the cellular proliferation and the recovery of the epithelial cells. Moreover, the differentiation process occurring in the cells of the upper part of the crypt, induces the synthesis of enzymes then localized in the brush border and involved in the membrane digestion process by which tri- and dimers are hydrolized to monosaccharides and aminoacids and then absorbed.

Many studies have been previously carried out using single session irradiations with doses between 200 and 1200 rad.

Multiple daily fractionations (MDF) have been studied in the present research to determine the tolerance of healthy tissues at high doses administered in a limited time interval.

The relevance of this study as far as concerns the Radioprotection Programme appears obvious since the biological test is a tissue of mammals and the use of MDF allows to investigate the mechanisms of damage induction and specially the phase of cellular recovery and return to a normal functional activity.

Two types of MDF were used in the present study; 200 rad fractions have been administered up to a 600 or 1200 rad dose. An interval of 8 hrs between the fractions was used in the first experiment; for the second experiment three fractions were administered every 4 hrs and then other three fractions were administered after 16 hrs in the 1200 rad total dose.

Female Wistar rats ageing 10-12 weeks and maintained at a rigorous L/D cycle from 6.30 a.m. to 6.30 p.m. were used in all studies. Irradiation was performed by a Telecobalt unit under anaesthesia only on the abdominal region.

Animals were sacrificed in all experiments at 10 intervals ranging from 1 hr to 29 days after the last fraction.

The biochemical parameters analyzed were: 1) activities of brush border enzymes, such as disaccharidases and dipeptidases synthetized by the epithelial cells during the differentiation process and whose modifications show the functional damage of cells; 2) activities of lysosomal enzymes as index of cell and tissue damage; 3) protein content.

Morphological observations were made in order to evaluate the cell modifications appearing at the different intervals after irradiation. Moreover, one hour before the sacrifice the animals were injected with 3H-Thymidine to evaluate cell kinetics parameters.

Since studies, also occurring in our laboratory, showed circadian oscillations of mitotic activity, uptake of 3H-Thymidine and enzyme activities typical of differentiated cells, we have considered if the beginning of the treatment at different hours of the day could induce different effects.

In fact our preliminary results, using a single dose, showed that different modifications are observed in the small intestine if the same dose was administered at different hours of the day.

In the 200x3x2 experiment with a fraction interval of 8 hrs, the treatment began at noon for a group of animals and at midnight for the second group.

Since the total time of irradiation was short, the MDF produced modifications quantitatively similar to the ones observed with a single dose.

The two 200x3x2 MDF showed a considerably lower damage than the one induced by an 800 rad dose; also the 200x3 fractionations showed lower damage than the one induced by a 500 rad dose.

Differences were observed also between the groups irradiated at midnight and at noon. The increase of brush border enzyme activities at the initial intervals is more marked in the "day" group. The lowest levels of activities are reached 12 hrs after the last fraction in both groups, but the return to normal values is faster in the "night" group. Lysosomal enzyme activities, very high during the damage phase, and protein content do not show significant differences between the two groups. Morphologic damage during the acute phase of the intestinal radiation syndrome is more marked in the "night" group: at the initial intervals a lack of mitoses is observed whereas in the "day" group the values are about 30% of controls.

Recovery processes appear more efficient in the "night" group whose intestinal epithelium more quickly returns to a normal morphology. This result is confirmed by the increased number of epithelial cells 72 hrs after the last fraction whereas this phenomenon is observed only at later intervals in the "day" group.

The study concerning the second MDF starting at night confirmed the good tolerance of the small intestine to these types of treatment. The total time for the treatment with 1200 rad has been in this group 8 hrs shorter than the one previously described.

The enzyme modifications in the two cases are similar enough even if the epithelium, at the initial intervals, appears less damaged than in the first MDF. The damage during the acute phase appears to be similar and, between 34 and 72 hrs, when the mucosa is deeply altered, the enzyme activities show values near to zero.

The return to a normal morphology is not coupled with the achievement of normal levels of the activity of functional enzymes, previously observed even after a single exposure, and appears more evident after MDF.

In fact, whereas the epithelium shows normal cells 5 days after the last fraction, at 11 days only the activities are similar to the controls.

All activities of the brush border show an overlapping behaviour thus confirming the previous results.

Lysosomal activities during the acute phase show a significant increase that appears higher than the one observed in the previous MDF, but even in this case the return to a normal morphology leads to normal levels.

Protein content shows similar modifications in both MDF.

Moreover the first results of the present experiment show that the 200x3 every 4 hrs MDF induces a more marked damage when compared with the every 8 hrs treatment.

The results obtained up to now show the good tolerance of this tissue with high proliferative activity with regard to total doses that, if administered in a single session, are able to kill 37% of the animals because of the gastrointestinal radiation syndrome.

Although the morphological and functional damages after MDF appear qualitatively and quantitatively similar to a single session irradiation with an equivalent dose, the repair and recovery processes result more efficient.

The number and the complexity of the considered parameters did not allow to complete the studies so that the conclusive results will be presented in the next year..

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Vertragspartner der Kommission:

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Nr. des Vertrags: 212-76-BIO B

Leiter der Forschungsgruppe:

Prof. Dr. J. Hüttermann Prof. Dr. A. Müller-Broich

Allgemeines Thema des Vertrags:

Studies of the effects of ionizing radiation on DNA and its constituents.

Titel des Projekts Nr. 1:

Optical properties of radiation induced free radicals in nucleic acid constituents

Leiter des Projekts und wissenschaftliche Mitarbeiter:

J. Hüttermann, H. Riederer

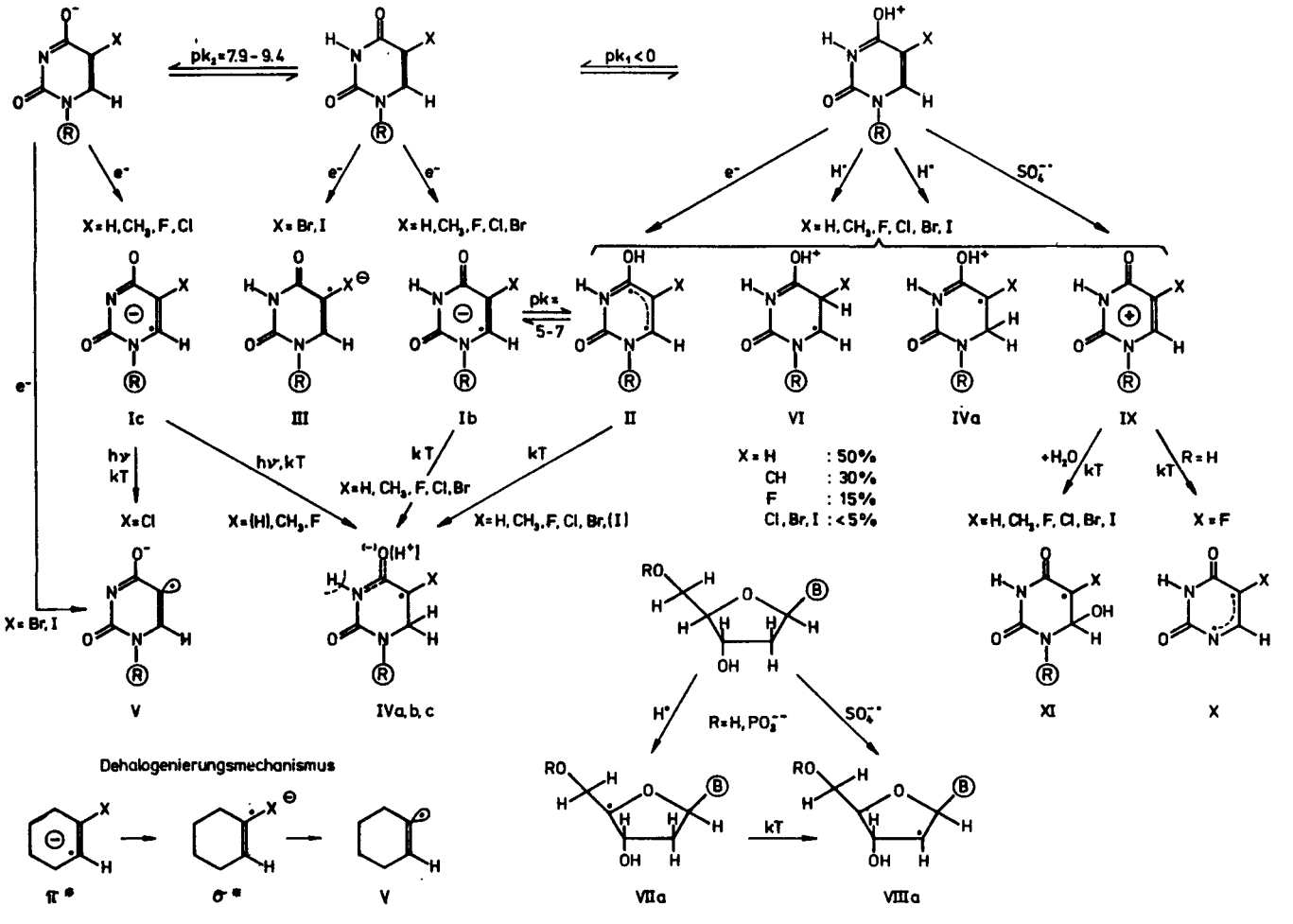
The project was aimed at the elucidation of optical properties of free radicals and their correlation to paramagnetic features by using combined optical and magnetic resonance spectroscopy as tools of investigation. In this way, the large body of data available from pulse radiolysis studies in aqueous solutions using optical detection methods should become relatable to the detailed structural information obtainable by ESR-spectroscopy on radiation-induced intermediates.

Specifically, the project delt with the study of the reactions of water-radiolysis products, $H^{\cdot}$, e, $OH^{\cdot}$ (and $SO_4^{-\cdot}$) in low-temperature glasses with uracil and its 5-methyl- and 5-halogen-substituted bases, nucleosides and -tides in order to elucidate the contribution of indirect radiation action in the in vivo DNA-radiosensitization occurring upon replacement of thymine residues by 5-halouracils in DNA. Glasses were chosen to facilitate both opti-

cal and ESR-detection of intermediates as well as the production of specific reactants allowing to study their reactions selectively. The glasses used were aqueous neutral (LiCl), acidic (H_2SO_4, H_3PO_4) or alkaline (NaOH) as well as organic (CH_3OH, MTHF) in nature. The solvent differences bring about different tautomeric forms of the solutes. The main reactions studied and their results are shown in the scheme where the solute bases are classified according to their tautomeric form. (The reactions with OH$^\cdot$ are still under investigation).

Of the three reactants studied, only H$^\cdot$-atoms and $SO_4^{-\cdot}$ anions, both produced in H_2SO_4-glasses either radio- or photolytically, react with deoxyribose in glasses containing the sugar or nucleosides (-tides) (bottom part of scheme). With H$^\cdot$-atoms, three radicals are formed, two of which are connected by a radical transformation; the more stable $C_{2'}$-radical is also formed by $SO_4^{-\cdot}$-attack. The latter species reacts with the bases (top right section) by electron transfer leading to a base cation; this can deprotonate at N_1, a process best documented for fluorouracil, or it can take up an OH^- at the C_6-position to yield a 5-yl radical. The latter process occurs in all nucleosides independent of the nature of the 5-substituent.

H$^\cdot$-atom reactions, which may be used as model for OH$^\cdot$-reactions, at the bases occur exclusively at the 5,6-double bond of the pyrimidine ring yielding 5-yl and 6-yl radicals, both of which are probably O_4-protonated in the extreme low pH of the acidic (H_2SO_4, H_3PO_4) glasses (pH $\simeq$ 0). It was found that the reaction mechanism differs from that in fluid solutions but is comparable to solid state reactions. Thus, in nucleosides (-tides) the relative yield of base to deoxyribose radicals which according to solution reaction constants should give a factor of 2-5 in favour of base radicals whereas the ratio obtained in the glass exhibits a slight preference for the deoxyribose (3:2). The same interpretation applies to the relative contribution of 5- and 6-yl base radicals in dependence of the 5-substituent. In solutions, the directivity of the substituent on the site of H$^\cdot$-addition is rather small whereas in the glass a strong preference for addition to the


pk₂=7.9-9.4
pk₁<0
X=H,CH₃,F,Cl
X=Br,I
X=H,CH₃,F,Cl,Br
X=H,CH₃,F,Cl,Br,I
pk= 5-7
Ic
III
Ib
II
VI
IVa
IX
hν kT
hν,kT
kT
X=Cl
X=(H),CH₃,F
X=H,CH₃,F,Cl,Br
X=H,CH₃,F,Cl,Br,(I)
X=H : 50%
CH : 30%
F : 15%
Cl,Br,I : <5%
+H₂O kT
R=H
X=H,CH₃,F,Cl,Br,I
X=F
X=Br,I
V
IVa,b,c
XI
X
Dehalogenierungsmechanismus
π*
σ*
V
R=H,PO₃⁻⁻
VIIa
VIIIa
kT

site adjacent to the substituent is observed if the latter is not a hydrogen. The relevant numbers are shown in the scheme (middle right section). It is important to note that the dominant radicals in 5-halouracils are of the 5-yl type which is stable vs. dehalogenation, again a difference to findings in aqueous solutions.

The electron reaction which only occurs at the bases is the most diversified one (left hand side of scheme), the result of attachment depending on the tautomeric form of the solute as well as on the 5-substituent. In acidic, the resulting pyrimidine anion is of the π^*-type for all substituents. This anion converts into a neutral 5-yl radical. For iodine, a σ^*-anion is formed in the neutral glass in which the electron is located in a σ^*-orbital of the carbon halogen bond; for bromuracil, this anion is formed in neutral glasses in addition to the π^*-type. This σ^*-anion could be the precursor to halide ion elimination. In alkaline glass, bromo- and iodosubstituted uracils immediately form halide ions plus uracil-yl σ-radicals whereas for chlorouracil an intermediate π^*-anion is observed which can be transformed into the uracil-yl radical by annealing or bleaching. For all other substituents, a π^*-anion is formed which converts, as in the acidic or neutral glasses, into a neutral 5-yl radical. The optical spectra could be correlated unequivocally to the free radicals described above for the cases of the π^*-pyrimidine anions (I) the hydrogen-addition radicals (IV) and (VI) and the base-cations (IX).

Due to space limitations, the bearing of the findings presented with respect to the radiation chemistry of DNA shall be outlined only in the context of the radiosensitization of DNA by 5-halouracil incorporation. The currently accepted model starts from electron-attachment to the specifically bromouracil base in DNA which yields bromide and a uracil-yl radical (V). The latter is then thought to initiate a strand-break by abstracting a hydrogen atom from a neighbouring deoxyribose. Our results indicate that halide elimination requires a σ^*-anion as precursor the formation of which occurs in sizeable fractions only in alkaline environment. At neutral pH in the low temperature glasses, the π^*-anion is formed more effectively and reacts in the same way as the corresponding species in thymine. It therefore appears that the halide-eliminatio

process is very sensitive to the environment and need not be functional in DNA if the reactions occurring in vivo relate more to the solid-state types than to the aqueous solution reactions.

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  in preparation

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  in preparation

Contractor: Hahn-Meitner-Institut für Kernforschung Berlin GmbH

Contract No.: 270-79-1 BIO D

General Subject of Contract: Investigation on the Radiation Damage in Biomolecules

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Head of Research Team and Coworkers: Prof.Dr.W.Schnabel, Dr.I.A.Raap, K.Washino, Dr.H.Kihara (in part), O.Denk (in part)

This work's aims were twofold:

(I) The quality of radiation damage in biopolymers should be studied by pulse radiolysis in conjunction with optical absorption and light scattering measurements,

(II) The influence of sensitizers and protecting agents on radiation-induced reactions of biopolymers should be investigated by these methods.

With respect to (I) prominence has been given to high energy radiation-induced oxidative degradation and aggregation processes, both of them leading to irreversible radiation damage.

Oxidative degradation processes were studied with single-stranded nucleic acids, such as poly(A), and with calf thymus DNA. From parameter studies (dose rate-, ionic strength-, and polymer concentration dependence) it was concluded that the rate of light scattering intensity change of a polymer solution, observed after irradiation with a 100 ns electron pulse is correlated to the reaction

$$2\ PO_2^{\cdot} \longrightarrow 2PO^{\cdot} + O_2 \qquad (1)$$

Reaction (1) is followed by a relatively rapid fragmentation reaction leading to main-chain rupture:

$$PO^{\cdot} \longrightarrow F_1^{\cdot} + F_2 \qquad (2)$$

At absorbed doses greater than ca. 10^3 rad the average number of radical sites per initial macromolecule is significantly greater than unity and processes according to (1) occur essentially as intramolecular reactions. Due to the inhomogeneous distribution of radical sites the reaction rates cannot be described by homogeneous kinetics in this case. In a theoretical treatise a stochastic model has been elaborated, therefore, for the combination of randomly produced radical sites on a polymer chain. This model takes into account rapid intramolecular radical-radical reactions according to a multiexponential decay, and subsequently occurring intermolecular reactions, as well as certain initial molecular weight and radical distributions.

Work concerning (II) yielded the following results: Crosslinking of native DNA, poly(A), poly(C), poly(U), and poly(G) was observed upon irradiating radiosensitizer-containing aqueous solutions. Sensitizers used were p-nitroacetophenone, nitrobenzene, N-ethylmaleimide and anti-5-nitro-2-furaldoxime. Crosslinking yields, as measured by the increase of the light scattering intensity after the pulse, were highest when OH-radicals were totally scavenged by t-butanol and solvated electrons were free to attack both the sensitizer and the polymer. The complicated mechanism is based on the indirect action of radiation involving radical ions of the sensitizer.

Investigations on the influence of protecting agents, such as cysteamine, on radiation-induced molecular weight changes of various polynucleotides and DNA are in progress at present.

Parallel to these studies, investigations with plasma proteins (albumin, immunoglobulin and fibrinogen) were carried out. Aggregation was observed. It occurs at a consequence of chemical alterations of the protein molecules, induced by the attack of OH radicals. The transient species formed during and a few μs after the pulse react within a period of several ms. At the end of this period the formation of aggregates commences. The primary radiation chemical process consists (among others) in the generation of nucleation sites, which subsequently promote aggregation.

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CONTRACTOR:	Max-Planck-Gesellschaft zur Förderung der Wissenschaften e.V.
CONTRACT NO:	214-76-7 BIO D
HEAD OF RESEARCH TEAM:	Prof. Dr. D. Schulte-Frohlinde Prof. Dr. C. von Sonntag
GENERAL SUBJECT OF CONTRACT:	Chemical and physicochemical studies of chain breaks in DNA induced by γ-irradiation

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Among the targets of radiation damage in the living cell DNA is considered as one of the most, if not the most important. Radiation-induced changes of DNA may involve alterations at the bases and at the sugar moiety. Often the latter can be directly connected with a DNA strand break. Besides these direct strand breaks alterations have been identified which turn into strand breaks if treated with alkali. They are termed alkali-labile sites. Radiation-induced alterations at the bases may also result in an alkali-labile site. Strand breaks and alkali-labile sites have been considered as quite important lesions.[1]

Any attempt to develop a scheme on radiation protection will need as a basis the understanding of the underlying molecular processes of radiation damage. In the preceding years we were mainly concerned to study the mechanism of formation of DNA strand breaks and alkali-labile sites in aqueous solution. There the major damaging agent is the OH radical, a radical which has also been considered to play a certain part in *in vivo* systems.[2]

The OH radicals are rather reactive; they add to the double bonds of the bases and abstract hydrogen atoms from the sugar moiety. Because of their high reactivity the latter reaction occurs more or less at random, i.e. sugar radicals with the free spin at all sugar carbon atoms are formed. Radicals which are less reactive than the OH radicals may abstract an H atom preferentially at C-1' (Research program 1977).[3] Subsequent to the attack at this position the base is lost and a DNA with a lactone structure at this position can be formed. This damage constitutes an alkali-labile site (Research program 1976).[4] A further alkali-labile site is generated, if C-2' is attacked in the presence of oxygen. As a result an erythrose unit (loss of C-1' and base) is formed which is still embedded in the not yet broken DNA chain (Research program 1977).[5] A number of reactions can start from the radical at C-4'. The phosphoric acid ester bond can be split (i.e. DNA strand break), thereby a radical cation is formed as an intermediate.[6,7,8] Ultimately broken DNA strands are formed which carry on one side a phosphate group (enzymatically clean) but on the other side an altered sugar (possibly enzymatically "dirty") (Research program 1979). The radical at C-5', in the presence of oxygen, leads to a DNA strand break by breaking the C-5' - C-4' bond. On one side of the broken DNA strand an altered sugar remains which again may be considered as an enzymatically dirty end. For a given set of enzymes (DNAse I, PDE I, PDE II, alkaline phosphatase) which degrade unirradiated DNA to nucleosides such structures may hamper the enzymes in their action. It has been shown (Research program 1978)[10] that the expected effect can be observed. However, the correlation between the identified end groups and the ineffectivity of these enzymes to work on irradiated DNA has not yet been substantiated (work in progress). Because of the importance of oxygen in the radiation-deactivation of living cells oxygen uptake measurements have been done with aqueous DNA and a large number of model compounds (Research program 1980).

It has been found that the purine/OH adduct radicals do not react efficiently with oxygen. From earlier work on GMP[11] one would have expected this for this compound but the ineffectivenes of the adenine/OH adduct radical was unexpected. A mixture of 5-'nucleotides equivalent to the composition of calf thymus DNA gave the same $G(O_2$ consumption) = 3.2 as has been calculated from the values of the individual 5'-nucleotides. However, calf thymus DNA itself showed a much higher $G(O_2$ consumption). Further, (partly single stranded) DNA shows a marked concentration, temperature and dose rate effect in $G(O_2$consumption) an effect also observed with RNA and carbowax 20 M [a polymer with the repeating subunit $-CH_2-CH_2-O-$]. The high yields in these polymers [e.g. DNA: $G(O_2$consumption) = 9.2 at a dose rate of 0.04 Wkg^{-1}, 50^{o} C, $4 \cdot 10^{-3}$M and carbowax 20 M : $G(O_2$ consumption) = 40 (similar conditions)] indicate chain reactions not observed with the low molecular weight material. One can speculate that a part of the strong sensitizing effect of O_2 may be due to such an enhancing effect although the effect with double stranded DNA appears to be not as marked as with partially denatured DNA. Any radiation protection will then have to take such chain reactions into account. Sulfhydyl compounds are known to protect against radiation damage. They also effectively interfere with autoxidation chain reactions.

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Contractor : University of Newcastle upon Tyne, England.
Contract nr : 226 - 76 - 9 BIO UK
Head of the research team : DR. G. SCHOLES

General subject of contract : Pulse Radiolysis of Aqueous Solutions of Purines and Related Compounds

Title of the project : Pulse Radiolysis of Aqueous Solutions of Purines and Related Compounds

Head of Project and Scientific Staff : Dr. G. Scholes, Dr. D.J. Deeble, Dr. A. Garner

An elucidation of the mechanism whereby DNA is degraded by OH radicals is of great importance with regard to an understanding of the indirect effects of radiation in vivo. DNA contains an equal number of pyrimidine and purine bases and although a fair amount is known about the interaction of OH radicals with pyrimidines, the situation is much less clear in the case of purines. This project has therefore involved (i) a study of the transient organic species produced on reaction of OH radicals with purines and related compounds by the technique of pulse radiolysis, using optical and conductimetric detection in the μs time scale (ii) an examination of the permanent products after stationary γ-ray radiolysis.

Purine solutions were saturated with N_2O prior to irradiation to ensure that OH is the major attacking species. Irradiations were also carried out with N_2O solutions containing oxidant, mainly $Fe(CN)_6^{3-}$, which can fix the site of radical attack by an electron-transfer reaction. The influence of the 'natural' oxidant, molecular oxygen, has also been investigated.

1. Adenine and Adenosine

In pulsed adenine solutions three radicals are distinguishable. One of these undergoes a first-order decay process ($k = 1.4 \times 10^5\ s^{-1}$), probably associated with ring-opening of a C(4) or C(5) OH adduct; the ring-opened form determines the complex nature of the final products. Radical interaction leads to some return of absorbance at 260 nm (λ_{max} for adenine) which is consistent with the reported low sensitivity of this particular purine to degradation in γ-irradiated solutions. There is also an optical absorption over the wavelength range 240 - 400 nm present after radical decay, remaining constant after several hundred milliseconds, and this is

probably associated with the precursor of a precipitate which is formed relatively slowly in these aqueous systems. The three radicals are oxidised by $Fe(CN)_6^{3-}$ and by $C(NO_2)_4$ with rate constants in the range 1×10^8 - 3×10^9 $dm^3\ mol^{-1}\ s^{-1}$; oxidation leads to a rapid increase in conductivity at pH's 5 - 6 ($t_{\frac{1}{2}} < 5\mu s$) consistent with proton release from the intermediate carbocation resulting from electron transfer from the radical to the oxidant. The presence of the oxidant suppresses formation of the precipitate and it is therefore likely that this is an aggregate of dimers formed as a result of interaction between adenine radicals. It is generally considered that the primary organic radicals produced by OH attack on a purine molecule react with oxygen to form peroxyradicals. It has been found that this reaction with oxygen is in fact quite slow; it was not observed in pulsed solutions of adenine so that the rate constant must be less than 10^7 $dm^3\ mol^{-1}\ s^{-1}$. Pulsed adenosine solutions show rather similar behaviour to those of adenine indicating that the radical reactions are only slightly modified by substitution at C(9).

In neutral solution in the absence of oxidant, G(-adenine) = 1.5 (G = molecules per 100 eV), G(8-hydroxyadenine) = 0.2 and G(dimer) $\underline{\sim}$ 0.5. With $Fe(CN)_6^{3-}$ present, G(-adenine) increases to 3.5 and there is an increase in the yields and number of products. The experiments indicate that $\sim$ 20% of the OH radicals react at C(8) in the imidazole ring and that the resulting radical is partly responsible in the restitution process in the absence of oxidant. The high value of $G(-Fe(CN)_6^{3-})$ = 15 indicates that this oxidant can undergo thermal oxidative reactions with intermediates leading to small molecular weight products.

2. Guanosine

Pulse studies of solutions of guanosine (owing to the limited solubility of guanine, experiments have been carried out with the more soluble nucleoside) indicate the formation of two organic radical species absorbing above 300 nm. Only one of these can be oxidized by $Fe(CN)_6^{3-}$, indicative of different oxidation potentials of the organic radicals. In the absence of oxidant there is a decrease in conductivity after the radiation pulse and a detailed study of the effect of pH has revealed that

the post-pulse conductivity drop follows a typical pK_a profile (pK_a = 3.92) with larger conductivity decreases at lower pH. These results are consistent with the scheme (Gu = guanosine):

$$Gu + .OH \longrightarrow .Gu(OH) \underset{-H^+}{\overset{+H^+}{\rightleftharpoons}} .GuOH_2^+$$

It should be noted that these particular conductivity changes are not observed in pulsed adenine or adenosine solutions over the same pH range; this may be a reflection of different pK_a values of the reaction intermediates.

In guanosine + $Fe(CN)_6^{3-}$ solutions over the pH range 4.5 - 5.5 there is a rapid increase in conductivity following the pulse, indicative of proton release subsequent to oxidation of the .G(OH) radical to the corresponding carbocation.

3. Hypoxanthine and inosine

Pulse-radiolysis studies of N_2O- saturated solutions of hypoxanthine and of inosine have been carried out. Only one radical ($\lambda_{max} \sim 340$ nm) is observed from hypoxanthine and this reacts rapidly with $Fe(CN)_6^{3-}$ to form an intermediate which then undergoes further acid-base catalysed first-order processes. The presence of the ribose moiety in inosine lowers the rate of reaction of the inosine OH-adduct with $Fe(CN)_6^{3-}$ - possibly due to steric factors - and increases, by two orders of magnitude, the subsequent solvent-initiated reactions. As with adenine, the hypoxanthine and inosine OH-adducts react too slowly with oxygen to be observed under the pulse conditions used.

In the absence of oxidant G(-hypoxanthine) =2. Products include 6-amino-5-formamido-4-hydroxypyrimidine (G = 1) and hypoxanthine hydrate (G = 0.5) indicating some attack on the imidazole ring of this particular purine. In the presence of oxidant the radiolytic decomposition of hypoxanthine is much less complicated than that of adenine and indicates the marked influence of the substituent at C(6). Thus, from hypoxanthine

irradiated in the presence of either $Fe(CN)_6^{3-}$ or oxygen, one major product is formed accounting for ∿ 75% of the purine decomposition yield (G = 4.5). In hypoxanthine + $Fe(CN)_6^{3-}$ solutions the oxidant reacts further with the minor products of the radiolysis. The major product from irradiated hypoxanthine /N_2O/O_2 solutions has the molecular formula, $C_5H_8N_4O_4$. (An earlier assignment of $C_4H_7N_2O_3$ is incorrect due to decomposition processes in the mass spectrometer). From experiments using specifically and non-specifically isotopically labelled hypoxanthine and from an analysis of the major product from inosine it is concluded that scission occurs in the imidazole ring. It is therefore suggested that the product is 5-amino-6-formamido-4,5,6-trihydroxypyrimidine which could formally arise from hydrolysis across N(7)-C(8) of the C(4)-C(5)-hypoxanthine glycol; this would indicate once more the vulnerability of the C(4)-C(5) bond in purines to OH radical attack.

4. Concluding Remarks

From the radiation-chemical standpoint an important aspect of radiation protection is a knowledge of the primary chemical lesions in biologically sensitive molecules, so that appropriate systems can be set up to either reverse or inhibit such lesions. The pulse-radiolysis studies of this project have given detailed information on the physico-chemical characteristics (optical absorption, decay kinetics) of the various purine OH-adducts, and the product analyses have given indications of the initial sites of OH attack. The structures of the organic radicals leading to specific products have yet to be elucidated.

K.D. Asmus, D.J. Deeble, A. Garner, K.M. Idriss Ali and G. Scholes:

Chemical Aspects of Radiosensitization Reaction of Sensitizers with Radicals Produced in the Radiolysis of Aqueous Solutions of Nucleic Acid Components. Brit. J. Cancer (1978) 37, Suppl.III, 46.

Contractant de la Commission : CEA, Grenoble
N° du Contrat : 158-76-9 BIOF

Chef du groupe de recherche : TEOULE Robert
Thème général du contrat : Etude des défauts formés au niveau des bases hétérocycliques par irradiation gamma de solutions aqueuses d'ADN ou de ses constituants élémentaires.

Titre du projet n° 1 : Etude des défauts formés au niveau des bases hétérocycliques par irradiation gamma de solutions aqueuses d'ADN ou de ses constituants élémentaires .

Chef du projet et collaborateurs scientifiques : M. BONICEL A., M. J. CADET et Mme N. MARIAGGI.

There is general agreement that DNA represents the essential target for ionizing radiation in living cells and there are many reasons to believe that the lesions of nucleobases are a source of mutagenesis lethality and carcinogenesis. The effort of our research group has been devoted to the determination of the chemical modifications induced on the base moiety under gamma irradiation. Other researchers of the Primary Effects Group are studying the final radiation products resulting from the damage of the deoxyribose moiety and the radical steps of the base degradation.

There are various types of lesions produced when DNA is irradiated and thus different final radiation products could be isolated such as free bases, altered bases and nucleosides, modfied bases remaining attached to the DNA polymer chain. Due to its high molecular weight it is difficult to assess the damage of DNA itself and prior to analysis the modified bases must be liberated from the DNA chain by mild acid hydrolysis. Analysis of the radiation products of the DNA constituents is however easier to perform and a lot of information has been gained from their spectroscopic studies (NMR and mass spectrometry). In addition, the radiation chemistry of free bases and free nucleosides has been a very useful guide to determine the nature of the final radiation products of DNA. It is worth nothing that our experiments dealing with the irradiation of DNA solutions have given results which were sometimes quantitatively and qualitatively different from those expected after

a simple extrapolation of the data provided by irradiation of the elementary constituents. In our research proposals we aimed at determing the chemical nature of the damage of adenine and thymine moiety in DNA. This goal has been attained and furthermore a detailed picture of the gamma radio-induced degradation of cytosine and thymidine has been obtained during these four last years.

The studies performed with the elementary constituents gave more detailed information on the mechanisms of degradation and the conformational properties of the radiation products. In frozen solutions experiments were carried out with thymidine. The final radiation products of thymidine were formed in very low yields. They were found to be identical to those obtained in solution at room temperature but, in addition, *threo* isomers, of the deoxyribose moiety and thymidine dimers were produced. Comparative studies of the oxidation of nucleic acid derivatives by OH radicals, singlet oxygen and superoxide anion radicals showed that guanine derivatives are the more sensitive to singlet oxygen and that superoxide anion radicals have a very small reactivity. The conformations of the various diastereoisomers of 5,6-dihydroxy-5,6 dihydrothymidine in aqueous solutions have been studied by ^{13}C and proton magnetic resonance spectrometry (CH_2OH rotamer, *syn* and *anti* conformation of the base, pseudorotational equilibrium of the deoxyribose residue.

In a first group of experiments DNA was selectively ^{14}C labelled on the thymine moiety and in a second group on the adenine moiety using *E. coli* auxotroph mutants. The DNA extracted by Marmur's procedure was irradiated in aerated and deaerated solutions. After irradiation the polymeric material was separated from the low molecular weight material by dialysis. The low molecular weight ^{14}C material was fractionnated by thin layer silica gel chromatography. It has been shown that the release of these products involves OH radical attack at the deoxyribose moiety and rupture of the N-glycosidic bond during the course of irradiation.

In a second step the high molecular weight material i.e. modified DNA chain was submitted to formic acic hydrolysis. The ^{14}C labelled products liberated from the DNA chain by this acid hydrolysis were separated by thin layer chromatography or high performance liquid chromatography. Due to

the small quantities available (10^{-9} g to 10^{-12} g in our experiments) characterization of radiation products was achieved mainly by comparison with reference samples using different solvents systems, adsorbents and column chromatography.

The radiation products liberated from the polymer during gamma irradiation of DNA solutions and those which remained attached at the DNA chain are listed in table 1.

Table 1 - Radiation products formed in the DNA chain by gamma irradiation of DNA solutions. A part of which could be liberated from the DNA chain during the time of the irradiation.

Base moiety	Aerated conditions	Deaerated conditions
Adenine	7,8-dihydro-8 oxo adenine 4,6-diamino-5-formamido-pyrimidine Urea Formyl urea	7,8-dihydro-8-oxo adenine 4,6-diamino-5-formamido-pyrimidine - -
Thymine	*cis* thymine glycol *trans* thymine glycol pyruvamide urea 5-hydroxy-5-methyl barbituric acid 5-hydroxymethyl-uracil	*cis* thymine glycol *trans* thymine glycol *cis* 6-hydroxy-5,6-dihydrothymine *trans* 6-hydroxy-5,6-dihydrothymine 5-hydroxy-5,6-dihydrothymine 5,6-dihydrothymine

In addition the presence of the N-formamido group in place of thymine after DNA irradiation as one of the major defects has been demonstrated. The formation of the N-formamido deoxyribose defect is due to the rapid decomposition of thymine peroxides produced inside the DNA chain by the irradiation. These thymine peroxides gave in equimolecular quantities carbon dioxide, pyruvamide and N-formamido-deoxyribole derivatives. Using an auxotroph mutant having its DNA selectively labelled at the C-2 of the thymine fragment the formamide defect could be easily evaluated by the measurement of $^{14}CO_2$ evolved. Evidence has been found that the cell constituents and the tertiary structure of DNA provide very efficient shielding and play a great role in the radioprotection of DNA bases. The 6-hydroxy-5,6 dihydrothymine defect formed in deaerated conditions is very labile in acidic medium. However it could be isolated by enzymic degradation of DNA chain.

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Contractor : The Kennedy Institute of Rheumatology

Contract nr : 271-79-1 BIO UK
Head of the research team(s) : G. Harris

General subject of contracts : The functional consequences of damage to eukaryotic cells by ionizing radiation

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Title of the project nr. 1 The functional consequences of damage to eukaryotic cells by ionizing radiation

Head of Project and scientific staff : G. Harris, I. Olsen, C. Russell and C. Browne

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The responses of lymphoid cells and tissues to ionizing radiation were studied during 1979 and 1980.

Organ cultures of rabbit spleen: The development of specific haemolysin-producing cells (PFC) in response to sheep red cells (SRC) in the culture medium was depressed by exposure to radiation at any time in culture. The most radio-sensitive period was at the time of most rapid increase of PFC in the cultures. Established PFC were highly radio-resistant with D_{37} value of around 10 Kilorads. In contrast to this, exposure of the tissues to less than 50 rads at 48 hours of culture, the period of peak response, had a definite depressive effect on the subsequent increase of PFC. This suppression was transient with doses <200 rads and was more permanent after greater exposures. In keeping with the radio-sensitivity of the development of PFC, DNA synthesis, but not RNA or protein synthesis was also found to be depressed by relatively low doses of radiation.

Further studies show that depressed (^{3}H) thymidine incorporation was not due to a pool effect resulting from competition by released precursors. Autoradiographic and molecular studies indicate that, following radiation, the cells in the exposed tissues were synthesizing less DNA. The nature of this altered DNA synthesis is now being elucidated and does not appear to be simply repair of damaged DNA.

These studies therefore show that the recruitment of cells into active antibody synthesis is highly radio-sensitive and depression of this process is associated with altered DNA synthesis. Excellent recovery followed exposure to doses of 200 rads, higher exposure produced more permanent damage.

Irradiation in hypoxic conditions not only protected the tissues from damage but also resulted in improved recovery from such damage. Speed of recovery was highest when the responses of the tissues to SRC were at their peak.

Post-irradiation studies of lymphoid cell lines: Human lymphoid cell lines were found to repair radiation-induced strand breaks, both rapidly and efficiently even after exposure to doses up to 30 Krads. After the DNA lesions were repaired, defective new DNA synthesis appeared. This was characterized by a persistence of the vulnerability of newly-synthesized, radio-labelled DNA to alkali, and was indicative of a slowing of the normal process of elongation and ligation of the new daughter strands. Significant changes were observed after relatively low doses of radiation around 300 rads, and they persisted at least until recovery of growth capacity had occurred in the surviving cells.

These results using two different systems of lymphoid culture thus indicate that significant effects can be measured following exposure to ionizing radiation at relatively low dose. They rely more on functional rather than structural assessment of macromolecular damage and could be a more sensitive means of assessing variation of individual radio-sensitivity at dosages which are more meaningful than heretofore.

Publications

Harris, G., Browne, C. & Cramp, W.A. (1981) The response of rabbit spleen explants to ionizing radiation. Int. J. Radiat. Biol. (in press).

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Contractor : W. Köhnlein, Münster
W.A. Cramp, London (Chairman of the cooperative research project)

Contract nr. : 227-76-7 BIO D

General subject of contract :

Post-irradiation changes at the cellular and sub-cellular levels.

Title of project nr. 1 : Post-irradiation changes at the cellular and sub-cellular levels.

Heads of Research Teams :
G. Ahnström, Wallenberg Labs. Univ. of Stockholm
P.E. Bryant, G.S.F., Frankfurt
W.A. Cramp, M.R.C., London
G. Harris, Kennedy Institute, London
W. Köhnlein, Westfälische University, Münster
M. Quintiliani, C.N.E.N., Roma.

The original intention of this group of workers was to collaborate, by meeting and by short visits between laboratories, in an attempt to correlate post-irradiation changes in DNA with cell death. Emphasis was to be made on such changes at low doses. Considerable effort has been made using the hydroxylapatite chromatographic technique to determine DNA lesions. At low doses we can summarise our results by saying that DNA breaks themselves do not closely correlate with cell death because the breaks initially induced by radiation are rapidly and extensively repaired. It is possible even with this sensitive technique that a small number of undetected breaks are responsible for cell death. However, our findings have prompted us to look for post-irradiation interactions between DNA and its neighbouring macromolecules which lead to lethality.

In this research we have very recently achieved some considerable success. Using as an end point the extent to which newly synthesized DNA is bound to its parent template, we have shown that radiation causes an increase in singlestrandedness due to lack of binding. This singlestrandedness becomes greater with time after irradiation, reaches a peak and then declines to unirradiated background levels. The extent to which this phenomenon occurs is dose and cell dependent.

We conclude that other macromolecules besides DNA mediate

in damage to DNA and that whilst DNA breaks are not a cause of cell death they may reflect the amount of energy deposited within the molecule. We have noted that in cells from patients suffering from ataxic diseases, which are more sensitive to the lethal effects of radiation, this phenomenon observed in newly synthesized DNA is much exaggerated. Whatever the mechanisms involved our findings suggest that by observing the quality of newly synthesized DNA, in peripheral blood lymphocytes stimulated into synthesis, a rapid screening procedure is available to determine person-to-person susceptibility to low doses of radiation. The exact nature of this DNA with respect to its strandedness is now being examined.

In the context of the Radiation Protection Programme we feel that we have made excellent progress in determining the nature of radiation damage and presenting a possible technique for assessing individual susceptibility to radiation.

The frequent meetings of small groups of research workers and the resultant cross-fertilisation of ideas has been very productive. Eighteen papers have been published or accepted and three more submitted. A productivity stimulated by the financial support derived from the contract.

1) "Extensive and equivalent repair in both resistant and sensitive E.coli determined by a DNA unwinding technique".
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British J. of Radiology, 50, 684-685, 1977.

2) "Further studies with hydroxyapatite detection of DNA strand breaks in bacteria and mammalian cells".
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Int. J. Radiat. Biol., 1980 (in press).

III. 5.

SOMATISCHE LANGZEITWIRKUNGEN IONISIERENDER STRAHLEN UND TOXIKOLOGIE DER RADIOAKTIVEN ELEMENTE

SOMATIC LONG-TERM EFFECTS OF IONIZING RADIATIONS AND TOXICOLOGY OF RADIOACTIVE ELEMENTS

EFFETS SOMATIQUES A LONG TERME DES RAYONNEMENTS IONISANTS ET TOXICOLOGIE DES ELEMENTS RADIOACTIFS

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Tätigkeitsbericht beschrieben : *

Further research work on these subjects will also be described in the following progress reports : *

D'autres travaux sur ce thème de recherche sont également décrits dans les rapports suivants : *

199-BIO N TNO, Rijswijk (Barendsen/Broerse)
215-BIO N KFA, Jülich (Feinendegen)
208-BIO D Univ. Würzburg (Kellerer)
248-BIO N Univ. Leiden (van der Eb)
203-BIO DK Finsen Institute, Copenhagen (Faber)
183-BIO EIR Techno. Dublin (Malone)
099-PSA F CEA-CEN, Fontenay-aux-Roses (Uzzan)
Biology Group Ispra

* Siehe auch Punkt IV,
See also section IV,
Voir aussi point IV,

Contractant de la Commission :
European Late Effects Project Group (EULEP)

N° du contrat : 201-76-1 BIO C
Chef du groupe de recherche :
Prof. J.F. DUPLAN

Thème général du contrat :
Late somatic effects of ionizing radiation in the mammalian organism.

EULEP has pursued its efforts during the years 1976-1980 on

a) standardization of experimental conditions in the participating institutions
b) coordination of the planning and performance of on going research projects in the areas of radiation late effects, and
c) on execution of specific cooperative projects on carcinogenesis, on dysplastic and dystrophic lesions and on the toxicity of internal emitters.

The activities of EULEP are subdivided in five committees :

1. Committee of Pathogenesis of Neoplastic Diseases
2. Committee of Pathogenesis of Non-Neoplastic Diseases
3. Committee of Internal Emitters
4. Committee of Pathology
5. Committee of Dosimetry for External Radiation.

Title of the Project 1: PATHOGENESIS OF NEOPLASTIC DISEASES

Head of the Project : H.J. SEIDEL

The Committee coordinated cancer research studies in 14 european laboratories. This work has been divided into 3 project areas :

1. Leukemia induction
2. Virus activation
3. Pathogenesis of solid tumors

In all the project areas, although rather different in the models and the techniques used, the same type of questions are asked. These concern primarily the "cells at risk", i.e. those cells which are transformed as a consequence of radiation exposure, their sensitivity for transformation by radiation of different qualities and - in leukemogenesis - their nature as determined by the possibility to prevent the disease. Also the role of viruses was studied in 2 models of tumor induction by radiation, leukemia after external radiation and bone tumor induction after incorporation of isotopes. Of special interest were experiments with autologous bone marrow transplantation in monkeys after total body irradiation which had been performed many years before and in which now the late effects - a high mortality of malignant tumors - are observed.

1. Leukemia induction

In 4 different laboratories it was investigated whether transplantable tumor cells arise shortly after leukemogenic treatment of mice by X-irradiation (4 x 1,75 Gy in weekly intervals) and persist during the long latency period. Leukemias of donor type were only seen in 2 or 4 Gy conditioned recipients when the donor mice already had manifest lymphoma. The genotype of these leukemias was clarified by use of a C57Bl mouse strain with 38 chromosomes.

In experiments on the prevention of radioleukemogenesis the best effects were obtained when the animals were transplanted with bone marrow cells from young donors (70 % incidence in controls was reduced to 13 %).

This inhibitory action has been ascribed to an early thymic repopulation after the leukemogenic radiation exposure by cells derived from the transplant - and indeed the results of a variety of protocols suggest a correlation between the protective effect and the ability of the transplant to provide thymic precursors.

Attempts are presently being made to identify the cell type in the bone marrow which is involved in the prevention of lymphosarcoma development. Specifically, the hypothesis is being tested that this cell is identical with the prethymic precursor cell in the bone marrow as described by Boersma et al.

Sequential changes in the cell surface of thymic cells during leukemogenesis were studied by biochemical methods. A diminution of sialopeptide in lymphoma cells was described and a loss of 4 galactose containing peptides. The induction of myeloid leukemias by a single exposure of CBA mice to different types of irradiation has been established as a reproducible model although with long latency periods and still low leukemia incidences. The main emphasis in these studies was given to the dose rate which was very high in the human exposures at Hiroshima and Nagasaki.

The synergistic action of radiation and a chemical leukemogen was studied using butylnitrosourea. Weekly doses from 6,25 to 100 rad were used. There was a significant enhancement of leukemia induction by 12 times 25 rad and also 12 times 12,5 rad, but a delay after 12 times 75 rad total exposure. The most effective leukemia induction was seen in mice exposed to the chemical after lethal irradiation and bone marrow transplantation, indicating a high susceptibility of regenerating hemopoiesis for leukemogenesis.

2. Virus activation

It is a known fact that viruses can be isolated from murine radiation induced leukemias. The nature of these viruses, especially of the RadLV-Rs

has been studied in detail - biologically, serologically and biochemically. For a better virus production leukemic extracts were propagated in young rats and an isolate called BL/F was obtained with a variety of leukemogenic properties. Separate pathogenic entities could be titrated and clonal isolates of a thymic lymphoma and a mixed cell lymphoma inducing agent were described. A series of molecular studies including hybridisation and restriction enzyme analysis led to the conclusion that the viruses generated as genetic recombinants between various C57Bl viruses represented in the RadLV-Rs complex and that each type of recombination results in the acquisition of a specific pathogenic potential. Related studies were also done using different cell culture methods and immunological approaches. In one laboratory these studies on viruses in radiation leukemogenesis were completed by parallel studies in a model of bone tumor induction in mice after incorporation of ^{224}Ra.

3. Pathogenesis of solid tumors

In studies on the induction of brain tumors in rats cells of the subependymal region were considered as cell at risk population. Their kinetics have been characterized in detail in steady state conditions and after irradiation and also after castration, since the glioma incidence had been significantly reduced after this procedure. This model is further studied in experiments using the implantation of benzpyren and subsequent single high dose irradiation.

Lung tumors were induced in mice after uniform irradiation of the thorax or non-uniformly using grids. One of the questions was whether it is justifiable to use average tissue doses to make risk estimates for carcinogenicity and was answered in the way that this crucial factor must be the dose received by the irradiated tissue. In the same model the tumor induction by cyclotron neutrons was compared to X-ray induction with a resulting RBE for tumor incidence of 1,7 - 5. A kinetic analysis of several lung cell types was performed after irradiation with 2 to 15 Gy. The most pronounced effects were seen in pulmonary alveolar macrophages and the least in endothelial cells. After 2 and 5 Gy a prolonged reduction of the proliferative

activity was seen in bronchial epithelial cells. Most interest now goes to the macrophages and the proliferation of their precursors.

With accurate dosimetry, it was possible to irradiate either one lung or two lungs only, and under the first condition the co-carcinogenic action of urethane will be investigated quantitatively in the same animal by comparison of the incidence and rates of the diagnosed tumors in the lungs separately. Results are not yet complete.

In a further model the induction of mammary gland tumors by radiation in 3 different strains of rats with different spontaneous tumor incidences is studied. By transplantation of mammary tissue, irradiated with radiation of different LET, the interactions between hormones and ionizing radiation will be analysed,using as recipients rats with elevated plasma oestrogen levels or a normal hormonal status.

The preliminary results from this study have shown that the technique of in vivo irradiation and transplantation of mammary tissue to ecotopic site was successful and reproducible, but the low number of tumors found in grafted mammary tissue may be attributable to the relatively short observation period chosen in the design of this experiment. In future complementary studies on mammary carcinogenesis at the tissue level, longer observation periods following transplantation will be obligatory.

Although the final analysis of the data is not yet complete, it can be concluded that the mouse ovary is confirmed to be a very sensitive organ to neoplastic transformation, with very little influence, if any, of the general conditions of tumor expression (in situ vs. transplantation), and high incidence at relatively low doses. It is also clear that, as it was shown for the bone-marrow, radiation can in fact induce immediate events of cell transformation to "potentially neoplastic progenitor cells", since transplantation after irradiation does not substantially change the shape of the dose-effect relationship, within the entire range of doses tested.

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Title of the Project 2: PATHOGENESIS OF NON-NEOPLASTIC DISEASES

Head of the Project : J.W. HOPEWELL

Over the period 1976-80 the efforts of the work of this committee have been concentrated on two important questions. The first of these has been to evaluate the importance of vascular damage in the development of late radiation effects. The multidisciplinary approach adopted by a number of members of this Committee to this problem in the rat brain has proved extremely valuable. However, towards the end of the present quinquennium, additional studies were initiated, using the microvasculature of the cheek pouch, in an attempt to establish a cellular basis for vascular changes. The second important question late radiation fibrosis has been carefully studied in the lung.

The role of vascular damage in the development of late effects in the irradiated rat brain (Project leader : A. Keyeux)

The effect of a single X-ray dose of 2000 rad to the whole brain was evaluated as this was considered to be equivalent to the therapeutic range used in brain irradiation. However, doses above and below 2000 rad were also investigated. The effect of radiation on the brain angioarchitecture was studied using a specifically developed vessel filling method (Reinhold, Rijswijk). The investigations showed that after a dose of 2000 rad, focal abnormalities mainly appeared in the central regions of the brain after an average latent period of 17 months. Investigation using the thick cleared specimens of brain have suggested that similar vascular changes develop with doses of 2500 - 3000 rad, although after a shorter latent period. Preliminary data indicate that changes may develop as early as 8 months after these higher doses and that most animals die after a latent period of less than 12 months. The brains of animals dying after a latent period of < 12 months show evidence of extensive white matter necrosis.

Examination of histological sections of rat brain after irradiation with single doses of 2000 and 2500 rad (Hopewell, Oxford) indicates that the type of lesions produces is a function of the exposure dose. This suggestion is supported by the effects observed after a dose of 3000 rad. Extensive necrotic lesions were found exclusively in white matter after a latent period of approximately 8-12 months. The fimbria hippocampus, internal capsule and corpus callosum were frequently involved. These histological observations which confirm and complement those on the modifications in the angioarchitecture suggest two types of lesion : the early delayed white matter changes developing after a shorter latent period but require higher doses (> 2000 rad) than that needed to produce late vascular effects (< 2000 rad).

The vascular changes in the irradiated brain were further investigated by quantitative morphometric methods (Wilkinson, Hopewell - Oxford). The cerebral cortex of brains irradiated with doses of approximately 2000 rad (1770, 2000 and 2200 rad) showed that at intervals of 3 and 6 months after irradiation there was a significant reduction in the vessel density in the cortex. However, after 9 and 15 months the vascular density was increased. In conjunction with these morphological and morphometric investigations, physiological eveluation of the radio-induced changes in the microcirculation of the brain were also performed (Keyeux, Brussels). By means of a radioisotope dilution technique based on the well differentiated behaviour of both (^{99m}Tc) sodium pertechnetate and (^{131}I) iodo-antipyrine in the brain, the cerebral blood volume, cerebral blood flow and the cerebral distribution space of iodo-antipyrine were evaluated in brain irradiated with 2000 rad. An increase in the cerebral blood volume was found after 6 months and was particularly significant 18 months after exposure. The increase in cerebral blood volume plus the comparatively large increase in cerebral blood flow was the reason for the substantial decrease in the circulatory mean transit time from 6 months after irradiation. The cerebral distribution space of iodo-antipyrine appears to increase linearly with age. Radiation appears to limit the increase in value of this function at about 6 months after exposure. Later the brain content of iodo-antipurine was lower in irradiated than in non-irradiated animals of the same age. At 18 months, the depression of the cerebral distribution space was emphasized while the increase in cerebral blood volume was lower when compared with that observed at 12 months.

Although an evident discrepancy exists for measurement performed 6 months after irradiation, the increase of the cerebral blood volume observed at periods greater than 6 months after exposure was in good agreement with the increase in vascular density reported in similarly irradiated rats. The cerebral blood flow, as evaluated in this study, was related to the total brain blood flow and not to nutritive flow only. The reduction of the cerebral distribution space after a latent period of approximately 6 months suggests that irradiation damages the cerebral capillaries in such a way that normal exchanges between blood and tissues was impaired. In this context, the corresponding increase of the cerebral blood volume could be interpreted as a consequence of capillary destruction. Other consequences of the direct and indirect late effects of radiation on the brain were approached by the characterization of some biochemical parameters which are related to different functions and structure of the brain (Gerber, Mol). Changes were followed from one day to 24 months after local exposure to 2000, 3000, 4000 and 6000 rad. Amongst the most important alterations observed were an increase in serotonine during the early (up to 3 months) and intermediate (up to 12 months) period and a temporary increase in DNA content during the intermediate period, (this was not confirmed by DNA synthesis studies in the cells of the brain). A dose dependent increase in collagen was observed from 3 to 18 months after exposure, sialic acid was decreased and alterations in uptake of alpha-aminoisobutyrate were observed.

In the silent period (the first months after irradiation), when morphological vascular alterations were not prominent, there were already possible changes in nervous function (serotonin), in myelin (sialic acid) and fibrosis may commence. No change in biochemical parameters characterizing endothelial function could be discerned up to 12 months after 3000 - 4000 rad.

Investigations of brain ultrastructure (Reyners, Maisin - Mol) were devoted to the study of the changes occurring in the glial cell population in the cerebral cortex. For doses greater than 1100 rad, a sharp drop in the number of glial cells, which are satellite to neurons, was observed by 15 days after irradiation. This effect was found to be caused by the specific depletion of

astrocytes with a very irregular nucleus. In most animals these cells appear to be able to repopulate although in a few they were still considerably depleted after 12 months.

It is difficult to correlate those transient "early" changes in glial cells with the other physiological and biochemical data. However, it is of great interest that the type of astrocyte under consideration are often encountered in close association with the outer wall of small blood vessels. Therefore, this cell loss could be the cause of the physiological disturbances observed in the microcirculation. This hypothesis might also provide an anatomical basis for the reduction in iodo-antipyrine extraction ratio in the brain after 3 months, although the observed reduction in vascular density seems a more likely explanation. On the other hand, the persistence of a modified vessel micro-environment could be related to the late appearance of teleangiectasis. It should be remembered that the above mentioned glial modifications were observed in the cerebral cortex and therefore cannot with certainty be extrapolated to the rest of the brain. Blood vessel changes are frequently found in the mid-brain and are only occasionally observed in the cerebral cortex.

In conclusion, the histological appearance of lesion in the brain are a function of the irradiation dose. An indirect mode-involving the microcirculation characterises doses less than 2000 rad and a direct mode involving the glia would characterise doses higher than 2000 rad. This statement is a partial response to the original question of this project : "How are microcirculation changes involved in the pathgenesis of late effects in the brain ?" According to these considerations, it becomes evident that damage to the cerebral microcirculation is likely to be the origin of brain radionecrosis developing in patient treated by intensive radiotherapy for brain tumours.

A cellular basis for late radiation damage to blood vessels
(Project leader : Dr Y. Gunn)

This study involves the collaboration of Gun - Oxford and Calvo - Ulm. Its aim is to establish a firm cellular basis for the development of late radiation

damage to the vascular system using the relatively simple vascular network of the hamster cheek pouch. Parallel morphometric studies have indicated a reduction in the size of the vascular bed 3 months after irradiation. This is a primary vascular response that is believed to be important in the aetiology of late damage. The present cellular studies have been oriented to examine changes in this time period after irradiation.

A single cheek pouch in sixteen-week old male Syrian hamsters was irradiated with doses of 2000, 2250, or 2500 rad. Three months after irradiation the animals were divided into two groups. One group was injected with ^{3}H-thymidine; injections were given twice daily, directly into each cheek pouch, for 3 days. Animals were then killed by perfusion fixation, the tissues processed and autoradiographs prepared. The animals in the second group were also sacrificed by perfusion fixation and the cheek pouches stained for nerve fibres with the Castro modification of the silver nitrate method of Cajal.

The preliminary observations on the nerve stained preparations have shown numerous small morphological changes. These consist of irregularities in the thickness of nerve fibres. Changes were not specifically located at the level of vessel dilatation but were generally distributed throughout the preparation. Hence no direct relationship between nerve damage and vessel dilatation can be postulated. Blood vessels may be influence by damage to distant nerve fibres.

These data are preliminary, and more is required before the mechanisms responsible for vascular changes can be fully evaluated.

Connective tissue changes in irradiated lung
(Project leader : Dr G.B. Gerber)

Lung has been chosen as a model to study the mechanisms responsible for the development of radiation fibrosis. Pulmonary fibrosis is a possible risk following radiotherapy of the thorax. The studies were carried on along several lines. Defining the biochemical and morphological alterations related to late lung damage (Mol), determining the conditions under which such damage occurs (Mol)

and characterising the alterations in connective tissue collagen induced by irradiation (Warsaw).

Long-term survival studies were carried out by Maisin (Mol) using BALB/c mice. Both lungs were exposed to single (20 - 80 Gy) or a fractionated dose (4 - 16 fractions of 2.5 or 5 Gy at 7 days intervals). Mice exposed to single doses showed a dose dependent survival time. Death was due to severe radiation pneumonitis with doses up to 60 Gy, but with 60 Gy or more early deaths were presumably due to oesophageal damage. Mice exposed to weekly fractions of 5 Gy, had a survival time dependent of dose (168 days) and died from radiation pneumonitis. The lesions that lead to early death were repaired under these irradiation conditions. Fractionation with 2.5 Gy per week again result in a dose dependent survival time which was much longer than after single exposure. This indicates that the damage leading to late lung lesions had, in part, been repaired.

Different biochemical and physiological aspects of radiation effects in lung were studied by Gerber (Mol) in cooperation with Warsaw. The most important alterations found after local exposure of rats to 10, 15 or 30 Gy were changes in fibrinolytic activity, in phospholipids, in collagen content and in blood flow. The work was, therefore, focused to the elucidation of the mechanism reponsible for these alterations. Techniques were developed to determine content and synthesis of the different types of collagen. The modified fibrinolytic activity was shown to result from a reduction in plasminogen activator in the blood vessel wall. Alterations in endothelial functions were investigated on the basis of specific functions, such as angiotensin conversion using isolated perfused lung. Other studies dealt with the possible prevention of fibrosis by the application of substances that interfere with collagen synthesis and blood clotting.

Chemical alterations in collagen were studied by Dancewicz (Warsaw). Collagen, irradiated with 50-500 Gy in de-aerated solution aggregates to dimers and tetramers and eventually polymers of tropocollagen as shown by sedimentation and light scattering methods. Tritium exchange and de-naturation kinetic studies indicate that the helical parts of collagen were diminished. A particular type

of fluorescence, due to dityrosine, appears in irradiated collagen. The mechanisms, by which dityrosine was formed were studied in irradiated tyrosine, glycyl-tyrosine, polytyrosine and protein (insulin, ribonuclease, papain, collagen solution). Collagen isolated from the skin of irradiated rats contains more dityrosine than that found in normal rak skin. Therefore, dityrosine formation may play a part in the insolubization of collagen e.g. in radiofibrosis. In irradiated lung, collagen III disappears in favour of collagen I, 6 months after a dose of 25 Gy.

Collagen irradiated in vitro displays slight changes in its antigenic properties. Mixed aggregates may also be formed between collagen and other proteins, and this was studied using the enzymatic reaction of tissue transglutaminase between fibrinogen and de-natured collagen as substrates. The results of these investigations suggest that radiation-induced changes in collagen, such as the formation of insoluble aggregates in collagen and fibrinogen, could provide a basis for the fibrotic process.

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Title of the Project 3: INTERNAL EMITTERS

Head of the Project : V. VOLF

Investigations were carried out on the effects of selected radionuclides (RN) in the body and the characterization of these effects in relation to the macroscopic and microscopic distribution of absorbed dose. Furthermore, the removal of incorporated RN from the body was studied as a means of preventing or reducing late radiation damage. Ten laboratories participated in these investigations, seven of which received some EULEP financial support. There have been four research projects, each convened by a project leader :

1. Late effects of bone seeking radionuclides
(Participating laboratories : MRC- and NRPB-Harwell, Karlsruhe, Mol, München)

Work on bone concentrated on radium and plutonium with the aim of estimating the osteosarcoma risk at low irradiation dose levels. Incorporation experiments in mice with short-lived bone-seekers such as ^{224}Ra, ^{227}Th and ^{177}Lu proved to be a promising tool for the study of dose response as a function of time. Protracted administration caused a significantly higher osteosarcoma incidence than the same activity given as a single dose (Moller and Ebert, 1978). These findings help to explain the unexpected occurrence of bone tumors in man after medical application of the short-lived ^{224}Ra, which was originally assumed to be much less toxic than the long-lived ^{226}Ra.

Following work done earlier on tumors produced by ^{239}Pu in laboratory rodents and on the gross distribution of ^{239}Pu in different bones, interest has centered on the micro-distribution of plutonium studied using special techniques of quantitative microautoradiography (Polig, 1978).

Interlaboratory consultations and a workshop on the technical and methodological aspects of localized dosimetry and quantitative histology in bone (Harwell 1980) helped workers to exchange views and experience in this promising new field of research. The results achieved were summarized in the EULEP symposium on bone (Rotterdam 1980).

2. Late effects after tritium incorporation
(Participating laboratories : Casaccia, London, Ulm, Warsaw)

In experiments on mice tritium has been administered as tritiated water, tritiated amino acids and totally tritiated diet ; the various sorts of intake more exactly model the predicted intake of man living in a tritiated environment. A long term toxicity experiment was concerned with "fully tritiated" mice which were produced by continuous exposure of pregnant animals, so that at parturition all the cells of their off-spring carried DNA with a tritium label. It was shown that newborn animals are at greatest risk, as assessed by uptake into DNA, but that only after exposure to high concentrations of tritium in drinking water were the weight gain and early development impaired significantly. Due to subsequent dilution and loss of tritium by cell division and migration the mice, although subjected to a very nearly whole body dose during development in utero, are irradiated subsequently in an heterogenous manner (Lambert et al., 1980). Furthermore, studies were performed on the blood stem cells as a possible biological indicator of radiation effects on the hemopoietic system from incorporated RN, or low doses of external radiation. Definite effects were seen following rather low tritium concentrations in drinking water, resulting in a whole body dose of about 1 rad per day (Harris and Fliedner, 1979).

At a scientific seminar on the metabolism and toxicity of tritium (London 1980) the research work done both in EULEP and EURATOM laboratories was summarised and there was a round table discussion on future trends in tritium research. Abstracts of the papers will appear in EULEP Newsletter.

3. Decorporation of Radionuclides and Late Effects
(Participating laboratories : NRPB-Harwell, Karlsruhe, Mol, Warsaw)

Methods and means for the therapeutic removal of RN from the body have been investigated. Experimental work on small laboratory rodens was concerned mainly with plutonium and other actinides as well as with uranium and radium. Removal screening studies included those with low

toxicity chelating agents such as Zn-DTPA, with newly synthetized chelons and their derivatives such as Puchel or DTPA-phosphonates or with chelates combinations such as DTPA plus desferrioxamine (NRPB Reports 1977 and 1978, Szot et al., 1978). For better prediction of the effect in man, dose- and time-effect investigations were carried out which enabled the efficacy of treatment to be determined more exactly. Treatment scheduling experiments were also performed to simulate the conditions likely to be encountered in clinical practice, such as prompt or delayed, single or protracted, local or systemic treatments (Volf, 1978).

Reduction of radiotoxicity provides the only direct evidence of the desired end-effect of radioactivity removal. Therefore, the effect of treatment with sodium alginate on the radiation damage to the hemopoietic bone marrow cells and to osteoblasts has been investigated : Using the spleen colony technique as indicator, a damage reduction factor of about 1.3 for bone marrow was estimated (Schoeters et al., 1980). The possible secondary effects of decorporation treatment were also studied. For example, after continuous administration of sodium alginates over a period of nine months, no interference with calcium and iron metabolism was noticed in mice.

Current research results in this area were summarized during a scientific seminar on the decorporation of RN (Karlsruhe 1978). The abstracts of the papers presented appeared in the EULEP Newsletter.

4. Late effects after inhalation of radionuclides
(Participating laboratories : MRC- and NRPB-Harwell, London, Mol)

The behaviour and effects of selected, mainly insoluble, alpha-particle emitting nuclides introduced into the respiratory tract were investigated. In mice, the initial deposition and late translocation of inhaled $^{239}PuO_2$ aerosols of different particle size have been followed. Further, the histopathology of the lung tissue, the effects on alveolar macrophages, the development of fibrosis and the microdosimetry of inhaled particles were studied (NRPB Reports 1977 and 1978).

The so-called hot particle problem (the suggestion that discrete insoluble radioactive particles might be more carcinogenic than when the same activity is more uniformly distributed) was studied using fission fragments generated from inhaled $^{235}UO_2$ and slow neutron exposure (Green et al., 1978). Various aspects of the clearance of radioactive and non-radioactive particles in rodents have been investigated in order to try to explain and predict radioactive damage (Gore and Patrick, 1978).

Progress reports concerning RN and lungs have been presented to both the "Internal Emitters" and "Non-Neoplastic Effects" Committee. Thus, the most competent scientific judgement can be provided on the work dealing with inhalation physiology and behaviour of inhaled RN as well as on the effects of radiation on the lung tissue. There has been a close contact between the two committees and common meetings have been organized, including a seminar on inhalation of RN (Harwell, 1978), EULEP symposium on pathophysiology of the lung after irradiation and other environmental factors (Reisensburg 1979) and a EULEP Lung Workshop (Harwell 1980). Minutes appeared in EULEP Newsletter.

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Title of the Project 4: PATHOLOGY

Head of the Project : C.F. HOLLANDER

The main activities of the Committee were directed towards standardizing the terminology used by the pathologists of the different laboratories working in EULEP in order to facilitate the comparison of results of experiments conducted in these laboratories. This goal is being achieved by holding topic-oriented workshops at regular intervals. These workshops also provide an excellent opportunity for some postgraduate teaching in order to maintain a high standard amongst the pathologists working within the framework of EULEP.

The results of these workshops are published in the EULEP Pathology Atlas, the editorial board of which consists of : W. Gössner, Chairman ; A. Luz and C. Zurcher, secretaries ; C.F. Hollander, J.R. Maisin and A. Nilsson, members. The fascicles of the Atlas are prepared by members of the Committee. Table 1 provides information on the published fascicles as well as those in preparation. It is the aim to distribute this Atlas to the scientific community outside EULEP in due time.

Members of the Committee also participated in symposia organized within the framework of EULEP by other Committees. These symposia were : "Pathophysiology of the lung after irradiation and other environmental factors" (Reisensburg), "Bone and bone-seeking radionuclides" (Rotterdam), "Lung workshop" (Harwell) and "Radiation-induced leukemogenesis" (Bordeaux).

The Committee maintained a Consultation Center which is localized in the Institut für Allgemeine Pathologie und Pathologische Anatomie der Technischen Universität, Munich. The members of the Consultation Center are the same as those of the Editorial Board of the Atlas. When necessary, additional members are drafted from the membership of the Committee. The Consultation Center provides advice to members of the Committee on problem cases in which consultation by other pathologists is considered important before a final conclusion is given.

TABLE I

EULEP PATHOLOGY ATLAS

AUTHOR(S)	CHAPTER	STATUS
GÖSSNER, W.	BONE	PUBLISHED
HOLLANDER, C.F.	THYROID	PUBLISHED
VAN ZWIETEN, M.J., ZURCHER, C.	MURINE INTESTINAL INFECTIONS	PUBLISHED
CALVO, W., HOPEWELL, J.W.	NERVOUS SYSTEM	PUBLISHED
STEWART, H.L.	LYMPHORETICULAR TISSUES	PUBLISHED
STEWART, H.L.	LUNG	PUBLISHED
VAN ZWIETEN, M.J.	MAMMARY GLAND, RAT	READY FOR PRINTING
MALDAGUE, P.	KIDNEY	IN PREPARATION
ZURCHER, C.	REPRODUCTIVE SYSTEM	IN PREPARATION
LUZ, A.	MAMMARY GLAND, MOUSE	IN PREPARATION
ZURCHER, C. LUZ, A.	SKIN	IN PREPARATION

The above-mentioned activities have provided the members of the Committee with sets of slides of cases which have been discussed in the Committee and upon which agreement has been reached with regard to classification of the lesions. These slide sets, together with the cases available in the Consultation Center, provide references to a variety of neoplastic and nonneoplastic lesions of pathologists working in the EULEP Laboratories.

A workshop on mouse lymphoreticular tumors was organised by the Committee on November 21, 1980, in Munich. Three outstanding experts on this subject from outside of the EULEP were invited to participate in the workshop : viz. Prof. G.R.F. Krueger, Prof. H.-E. Schaefer and Dr G. Hoffman-Fezer.

The purpose of this meeting was to consider the usefulness of applying a variety of morphological and functional criteria to mouse lymphoreticular tumors, in order to develop a classification which better reflects the view that these tumors constitute tumors of the immune system. In the classification presently used only morphological parameters are employed in classifying these tumors.

During the morning session of the meeting, results obtained from a EULEP-supported, cooperative project between the laboratories in Rijswijk and Munich on this topic were discussed. Out of 20 cases investigated until now, 7 were selected for presentation in extenso. Light microscopic, electron microscopic, histochemical, immunofluorescence and serological data were integrated into a definitive diagnosis, combining morpbological as well as functional characteristics. During the afternoon session the following papers were presented :

1. Classification of malignant lymphoma of the mouse using morphological, immunological and cytochemical methods.
   - G.R.F. Krueger (Cologne)
2. Immunohistochemistry on mouse lymphoid tissue.
   - G. Hoffmann-Frezer (Munich)
3. Non-Hodgkin's lymphoma classification, according to Lukes and Collins, applied to reactive and neoplastic lesions of lymphoreticular tissue in mice and man.
   - C. Baroni/V. Covelli (Rome).

4. Immune responsiveness of aged chimaeras with reticulum cell sarcoma.
   - V. Covelli (Rome).

In addition, Professor H.-E. Schaefer (Cologne) gave a short presentation on differences in histochemical staining of hemopoietic cells from various rodent species.

Most discussants felt that investigations on immunological markers of B and T cells (including Ig classes, light and heavy chains and T subset markers) were helpful in the classification of lymphoreticular tumors in mice. They were regarded as high useful not only in order to understand basic mechanisms underlying the development of these tumors, but also as a prerequisite for the development of animal models of human diseases, as well as to allow intercomparison of results from different laboratories. This workshop was attended not only by members of the Committee but also by members of the other Committees of EULEP.

Finally, the above mentioned activities of the Committee also provide support for ongoing work in the laboratories of EULEP not mentioned in detail in this report. The educational value of the ongoing activities, in terms of wealth of material and subsequent expertise supplied by the larger pathology groups of the smaller groups within EULEP is immeasurable, in the sense that the smaller groups would not see such a broad spectrum of material within their normal work life.

Title of the Project 5: DOSIMETRY FOR EXTERNAL IRRADIATION

Head of the Project : J.J. BROERSE

A prerequisite for coordination of research programs on late biological effects of toxic agents, including ionizing radiation is the standardization of experimental methods and materials such as exposure arrangements and dosimetry, pathology and the quality of experimental animals.

During the past contract period the third and fourth EULEP X-ray dosimetry intercomparisons have been performed for total body irradiations of a mouse phantom. These intercomparisons were considered to be essential for a periodical check on the X-ray dosimetry procedures and for the benefit of new groups joining EULEP during the contract period. In accordance with previous procedures the intercomparisons were performed by using mailed thermoluminescent (TL) dosimeters. The participants were asked to irradiate a mouse phantom in such a way that the test capsule at the central position would receive an absorbed dose of 200 rad in soft tissue and the irradiation could be considered as uniform (a ratio of less than 1.15 between maximum and minimum absorbed dose). An HVL of at least 1.5 mm Cu was recommended. The procedure for handling the thermoluminescent material was comparable to that used during the former intercomparisons. The estimated precision and uncertainty in absorbed dose derived by the TLD system are about 1.2 percent and 3 percent, respectively. Considering these values for precision and overall uncertainty the following recommendations were formulated (Broerse, Zoetelief and Puite, 1978) :

a) The accuracy of the dosimetry is considered to be satisfactory when the mean value of the results from a laboratory differs by less than 5 percent from the standard value.
b) When a difference between 5 and 10 percent from the standard values exists, a discrepancy in the dosimetry is indicated.
c) If the difference is more than 10 percent, a recalibration of the X-ray dosimetry system is indicated.

The TLD readings from phantoms irradiated at the participating institutes have been compared with those irradiated at the standards laboratory. An evaluation

of the results of the 1971, 1973, 1976 and 1980 EULEP X-ray dosimetry intercomparisons is presented in table 1. It can be concluded that after the first and second intercomparison, considerable progress had been achieved with respect to the differences from the standard dose. It is disappointing that during the 1980 intercomparison, the results of 2 of the 12 participants showed deviations in excess of 10 percent. It can further be concluded that, between the first and second intercomparison sessions, considerable improvements have been made with regard to the homogeneity of the dose distribution, however, in this respect the subsequent intercomparisons do not indicate any progress. Site visits of members of the dosimetry committee revealed inconsistencies in dosimetry procedures at some institutes and systematic errors in dosimetry equipment employed locally. These failures in either ion chambers or electrometer devices, which would not have been detected otherwise, could easily introduce variations in the determination of the absorbed dose by 6 to 8 percent.

Late effect studies caused by the irradiation of specific organs, e.g., lung and liver, are included in the EULEP research program. For these studies collimated X-ray beams have to be employed, since it is necessary to restrict the dose to other essential organs in the animal. In connection with these programs an intercomparison of X-ray dose and dose distribution was performed in 1978 for partial body irradiation. The aim of this intercomparison was to obtain information about the adequacy of exposure arrangements, which will determine the dose distribution and about the absolute accuracy of the X-ray dosimetry performed at the different institutes. For this purpose two acrylic plastic rat phantoms for irradiation of the lung substitute and liver substitute part, were sent by mail to each of the eight participating institutes. The dose in the irradiated organs as well as the scattered doses in the phantom have been measured and compared with the dose values from a standardization laboratory.

The results of the partial body dosimetry intercomparison are given in table 2. Large discrepancies (up to 36 percent from the standard value) were observed for the doses delivered by the participants. For some institutes considerable

TABLE 1.

EVALUATION OF RESULTS OF THE 1971, 1973, 1976 AND 1980 EULEP X-RAY DOSIMETRY INTERCOMPARISONS WITH THE MOUSE PHANTOM

Difference from standard dose

	$\Delta D \leqslant 5\%$	$5\% < \Delta D \leqslant 10\%$	$\Delta D > 10\%$
1971	8/13	0/13	5/13
1973	10/14	3/14	1/14
1976	15/16	1/16	0/16
1980	6/12	4/12	2/12

Absorbed dose distribution over mouse phantom

	$D_{max}/D_{min} < 1.06$	< 1.10	< 1.15	> 1.15
1971	1/12	2/12	5/12	7/12
1973	1/13	5/13	10/13	3/13
1976	2/15	5/15	11/15	4/15
1980	2/12	4/12	6/12	6/12

TABLE 2.

RESULTS OF THE EULEP PARTIAL BODY DOSIMETRY INTERCOMPARISON WITH THE RAT PHANTOM

institute	focus to top phantom (FSD) in cm	measured dose / stated dose		gradient*	
		lung	liver	lung	liver
A	30,32	0.88	0.90	35	67
B**	24	0.98	0.98	2	4
C***	43,50	1.07	0.98	2	2
D	70	0.96	1.07	14	41
E	26	1.36	1.28	29	57
F	60	0.95	1.10	15	48
G	50	0.99	0.99	21	56
H	50	1.09	1.04	20	54

* gradient = $\left[(\text{top dose} - \text{bottom dose})/\text{central dose}\right] \times 100$

** phantom turned 180° halfway through irradiation

*** irradiation from top and bottom using two X-ray machines.

differences were determined between the doses given to the lung and liver substitute material. For six of the eight participating institutes the doses in lung and/or liver material differ more than 5 % from the standard dose. For unilateral irradiations dose gradients between 40 and 70 percent have been observed over the total depth of 3 cm of liver equivalent material. These inhomogeneities in dose distribution over an organ with these dimensions are unacceptable for radiobiological experiments.

After receiving the results of the dose intercomparisons the institutes A, C, D and E checked their dosimetry procedures and stated that they had discovered the cause of the discrepancy of their dose values. Some of the errors were rather elementary, such as incorrect exposure time, incorrect calculation of attenuation in the lung substitute material and neglect of energy dependence of their own TL dosimetry system.

The results of the X-ray dosimetry intercomparisons for total body irradiations of the mouse and partial body irradiations of the rat demonstrated the need for improvements in dosimetry procedures and for repeated intercomparisons at intervals of 2 to 3 years. A revised version of the EULEP protocol for X-ray dosimetry is in preparation

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Contractant de la Commission : Centre d'Etude de l'Energie Nucléaire Mol.

N° du contrat : 232-76-1 BIOB

Chef du Groupe de Recherche : J. MAISIN

Thème général du contrat : Late somatic effects, cancer induction and genetic radiosensitivity in mammals.

Titre du projet n° 1 : Late effects in irradiated lung.

Chef du projet et collaborateurs scientifiques :

G.B. Gerber, J. Deroo, P. De Preter.

Lung had been chosen as a model to study the mechanisms of fibrosis because pulmonary fibrosis is a grave risk after radiotherapy of the thorax. In a first series of experiments a dose of 3 krad was delivered to one half of the thorax of rats and the animals were followed for up to 9 months. The parameters studies were collagen, DNA, lysosomal enzymes, peroxides and fibrinolytic activity. The most important alterations observed were an increase in concentration of collagen starting from 4 months, a decrease in fibrinolytic activity during the entire postirradiation period, an increase in lysosomal enzymes, biogenic amines and DNA. In the next series of experiments, doses of 1 and 1.5 krad were delivered and in addition to the above parameters, blood flow by means of microspheres and lipid content and composition were followed. After these doses, the changes in collagen occured later (11 months after 1 krad), and the reduction in fibrinolytic activity could again be detected. Moreover, lipids and blood flow in irradiated lung were reduced. Modifications were also observed in sialic acid content and lysosomal enzymes. Investigations on mice,obtained from Dr Lindop (London) confirmed these observations and allowed to ascertain that mice are more radiosensitive than rats with respect to late lung damage.

Further work then focussed on the elucidation of the mechanisms of the three alterations considered most important in the genesis of fibrosis, fibrinolytic activity, blood flow and collagen synthesis. Techniques were developed to isolate native collagen from lung and to determine the content of the

different types of collagen. In addition, a method to determine the different glycosaminoglycans in lung connective tissue was set up. Cooperation with Argonne National Laboratory (U.S.) was initiated to study connective tissue changes in dogs exposed continuously to high doses of radiation up to 10 krad. Synthesis of collagen is determined by measuring incorporation after injection of labeled hydroxyproline in vivo as well as after incubating lung tissue in vitro. These experiments are still being continued. The reduced fibrinolytic activity which could be responsible for the organisation of the exudate was studied in tissue slices and by means of a histochemical method was shown to result from a reduction of plasminogen activator in the vessel wall after 1.5 krad. Since damage to endothelial cells may be responsible for vascular alterations techniques were elaborated to follow endothelial functions. To this end, lung perfusions were developed and specific endothelial functions are being investigated by means of this system. So far no alterations in activation of angiotensin has been found after irradiation to 1.5 krad.

Titre du projet n° 2 : Late effects in the irradiated central nervous system.

Chef du projet et collaborateurs scientifiques :
G.B. Gerber, J. Deroo, J. Maes.

Brain was chosen to study the interaction between vascular alterations and organ function because little changes from an altered cell replacement can be expected in this organ. A first series of experiments followed a large number of biochemical parameters in the brain of rats exposed to 2 krad to their head. The parameters studied were : DNA, protein, hydroxyproline, sialic acid, beta-glucuronidase, acid phosphatase, cathepsin, serotonin, noradrenaline, dopamine, g-aminobutyrate, histamine and uptake of a-aminoisobutyrate by brain and other organs determined. The most important alterations found were alterations in lysosomal enzymes and uptake of aminoisobutyrate particularly during the early and intermediate time, and a decrease in sialic acid during the entire postirradiation period. During the intermediate period from 3-9 months, an increase in serotonin was observed. In another experimental series, higher local doses of 3, 4 and 6 krad were studied and other parameters, peroxides, total and individual lipids and capture of microspheres (relative blood flow) were also investigated. After such doses, serotonin content was enhanced for a longer period of time and an increase in hydroxyproline was noted already after a few months. Changes in blood flow (an early depression and a later increase) were observed. The alterations induced by irradiation were compared to those caused by exposure to lead where an increased serotonin content was also found. In subsequent experiments, a technique based on the autoradiography of radioactive microspheres was developed to study regional blood flow, but in contrast to observations after lead treatment no significant alterations were so far found after irradiation. Other investigators of the EULEP CNS group reported alterations in vascular structure as measured by means of injected resin and of in vivo brain blood flow (measured by injection of pertechnetate and of iodoantipyrine).

Therefore, these techniques were adapted to be used in parallel with our microsphere techniques to study regional blood flow and a series of experiments has been started after different doses. A technique to isolate capillary fragments from brain has been further perfectioned. Alkaline phosphatase, glutamate transpeptidase (two characteristic enzymes of endothelial cells) as well as uptake of methyl d glucose were assayed in these preparations from 1 to 12 months following a local exposure to 3 or 4 krad.

Short term effects (up to one week) were also investigated in brain exposed to 10 or 15 krad of X-rays and no significant alterations in these parameters could be observed late after 3 or 4 krad. Only slight changes were observed one week after low doses. It is concluded that these endothelial functions are not greatly affected by such an irradiation.

The studies on biochemical indicators of radiation damage were also completed during this contract. After having elaborated methods to determine a series of potential indicators of radiation damage in urine, such standardized methods were also worked out for blood. Tests on these indicators in rats exposed to different whole and partial body doses showed that certain tests respond well to doses in the lethal dose range and somewhat below. Data on rats are, however, unsuitable to make extrapolation to man and it is hoped that human or primate samples be made available to continue these studies.

Other investigations related to very early effects of radiation which may be useful to recognize radiation damage pertained to changes in blood flow, extravascular space, renal excretion and, in particular, to the mechanisms by which a state of shock arises after high level irradiation. These investigations also dealt with the possibility to influence by pharmaceutical means such a shock.

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Titre du projet n° 3 : Relative biological effectiveness (RBE) of 50 MeV neutrons compared with ^{60}Co gamma rays in inducing leukemias and cancers in mice

Cher du projet et collaborateurs scientifiques :
J.R. Maisin, A. Wambersie, G. Mattelin,
M. Lambiet-Collier, J. Gueulette.

During the period 1976- 1980 we have (1) terminated our programme on chemical protection against the long term effects of whole-body exposure of mice to ionizing radiation and (2) we have started with our experimental programme on relative biological effectiveness (RBE) of 50 MeV neutrons compared with ^{60}Co gamma rays in inducing leukemias and cancers in mice.

I. Influence of chemical radioprotectors on long term survival and causes of death of X-irradiated mice

We will summarize the data we have obtained on

I.1. the protection offered by a mixture of 2-β-aminoethylisothiouronium-Br-HBr (AET), serotonine (5-HT), glutathione (GSH), mercaptoethylamine (MEA) and cysteine (Cyst) on the long term survival and causes of death of X-irradiated mice with a single or with fractionated exposure (ref.I 1-15

I.2. the search of preleukemic cells in the bone marrow of mice irradiated with four weekly doses of 1.75 Gy whole body X-irradiation (ref. II 1-2).

I.1.1. Long term survival

7373 BALB/c and C57B1 male mice, 4 and 12 weeks old (LD50/30, 5.76 and 6.50 Gy respectively) were exposed to X-rays in the range of 1 to 20 Gy. Mixtures of radioprotectors increase the degree of protection compared with that obtained with each substance given separately.

The dose-effect curves for the long term survival obtained for irradiated untreated mice and for mice treated with a mixture of radioprotectors are not parallel. Thus the dose reduction factor (DRF) varies with the X-ray dose administered. The optimum DRF obtained for long term survival of BALB/c mice (2.5) was very close to that obtained against acute effects of ionizing radiation (2.8).

I.1.2. Causes of death

All mice were followed until spontaneous death ; the lethal diseases were classified in 12 categories. The data were evaluated by the method of competitive risks. Death in nonirradiated BALB/c mice is largely caused by tumors, in particular by lung cancer and nonthymic lymphoma whereas other causes predominate in the C57Bl strain. Radiation-induced life shortening of nonprotected mice is due to increased and advanced incidences of specific diseases, mainly thymic lymphoma in the medium dose range and glomerulosclerosis in the high dose range. Thymic lymphoma in BALB/c mice increases to a maximum at 6.5 Gy and declines thereafter. A shorter latency period is found for lung carcinoma, although the absolute incidence decreases because of the earlier deaths of the irradiated mice. After AET treatment, the maximum incidence of thymic lymphoma in BALB/c mice is shifted to 10 Gy but its height is unaltered, whereas treatment with a mixture not only displaces the maximum to a still higher dose but also decreases its frequency. Protection in BALB/c mice is also possible although to a smaller extent, against myeloid leukemia, sarcoma, glomerulosclerosis and non cancerous lung lesions but it is protection against the two latter diseases which contributes most to the prolonged survival in the high dose range. The protection in C57Bl mice resembles that in the BALB/c strain. Thus protection is effective against thymic lymphoma (possibly also against liver adenoma, all carcinomas and myeloid leukemia) as well as against glomerulosclerosis and non-cancerous lung lesions.

I.1.3. In C57Bl strains of mice exposed to four fractionated doses delivered at weekly intervals (total dose from 4 x 0.5 and 4 x 3.75 Gy). Life shortening showed a sigmoid dependency on the dose in non protected and protected mice with a dose reduction factor of 2.1 at 50 % life shortening. Thymic lymphoma was the most predominant cause of death in irradiated C57Bl mice. Radioprotectors diminished significantly the incidence of this disease but apparently did not reduce other causes of death. Reticulum cell sarcoma B also increased at the same low doses as thymic lymphoma. Both thymic lymphoma and reticulum cell sarcoma B increased in frequency after a fractionated dose compared to a single exposure with doses of 6-7 Gy and 3 to 4 Gy respectively.

I.2. In order to elucidate the mechanisms of radiocarcinogenesis in mice, we have investigated whether tumor cells arise already shortly after leukemogenic treatment by X-irradiation and persist during the long latent period or whether they appear only as the tumor develops. A C57Bl strain of mice with 38 chromosomes, histocompatible with our C57Bl/Cnb mice, was selected to detect the presence of preleukemic cells in the bone marrow and the thymus of irradiated mice. C57Bl/Cnb male mice, 4 weeks old, were exposed to 4 doses of 1.75 Gy of whole-body X-irradiation given at weekly intervals. Seven, 14, 30, 60 and 90 days later, a suspension of 10^7 bone marrow cells or thymic cells was prepared from each C57Bl/Cnb mouse and injected intravenouslyinto 1 C57Bl/Rb(6;15)1 Ald male mice exposed or not to 2 Gy of X-rays. The lymphomas developing in the recipients were analysed with respect to their genotypes by determining the percentage of donor cells in metaphase in the bone marrow, thymus, lymph nodes and spleen of the host.

Our results so far suggest that no preleukemic cells occur in the bone marrow and the thymus during the preleukemic period. After completion of these still ongoing experiments, we intend to investigate whether leukemia induced by radiation is associated with alterations of chromosome 15 in mouse T cells.

II. Long term effects of whole body exposure of mice to single and fractionated doses of ^{60}Co gamma rays and 50 MeV neutrons

The aim of this study is to determine the relative biological effectiveness (RBE) of 50 MeV cyclotron-produced neutrons (0.02 to 3 G) compared to ^{60}Co gamma rays (0.25 to 6 G) for life shortening and for leukemia and cancer induction in mice. The sequeles of an exposure to single irradiation are compared with doses of irradiation given in 10 daily fractions in order to assess the amount of reparable and non-reparable lesions for both types of radiation. Two strains of mice, C57Bl and BALB/c mice, were selected for this study to investigate the influence of differences in spontaneous incidence of leukemia and cancer.

Today, some of the 5000 irradiated and control mice are still alive and the evaluation of all the data is not yet complete. This report concerns only the results on cumulative mortality and causes of death of mice which die within 815 days and which were irradiated with a single or with 10 daily fractions of ^{60}Co gamma rays.

II.1. Mean survival and cumulative mortality

No significative difference was observed for the mean survival time and the cumulative mortality between controls and irradiated mice exposed to single or fractionated gamma rays from 0.25 to 2 G. After higher exposure (4 to 6 G) survival was significantly shortened. Mice exposed to fractionated doses of 4 and 6 G have a longer mean survival than the mice exposed to the same single dose of gamma rays.

II.2. Causes of death

After single or fractionated irradiation from 0.25 to 2 G , no significative differences in leukemia incidence were observed between irradiated and control mice. After higher exposure doses, the incidence of all leukemias was significantly higher in irradiated mice compared to controls. In addition, the total incidence of leukemias was higher in mice treated with 4 and 6 G fractionated exposure, than after a single one. Leukemias after fractionated exposure appear later in life than after a single dose. This is due mainly to thymic lymphoma which was more pronounced after a single than after fractionated exposure. Conversely, more non thymic lymphoma are found after fractionated exposure.

Compared to controls, the incidence of carcinoma tend to increase after a single exposure between 0.5 and 2 G and to decrease after higher doses yielding an incidence below that in controls after 6 G. After fractionated exposure, no significative differences were observed.

The dose effect curves of all types of malignancies (leukemias and carcinomas) after exposure to a single dose of gamma rays display a progressive increase with maximum at 2 G. The increase was progressive and less marked and the maximum was reached in mice after fractionated irradiation only after an exposure to 6 G.

Conclusions

Our preliminary data do not reveal any life shortening by small doses. At doses of 4 and 6 G, life shortening was less pronounced after fractionated than after single exposure. Leukemia and cancer tended to be less frequent after fractionation of small doses but increased after large doses compared to a single one. In the latter case, leukemias and cancers mainly increase because the animals lived longer. These experiments are still in progress and these results have to be confirmed and completed when all data will be available.

The data obtained in the frame of this programme are of benefit to the European Community. The data on the protection offered by mixture of chemical protectors demonstrate that chemical protective drugs could in theory be used to protect individuals against the shortening of life span and the causes of death after whole-body irradiation. In addition, our data on the determination of RBE for 50 MeV neutrons for cancer induction will be of straight forward interest for radiation protection purposes because only scarce data exist at present for this radiation quality. From a more theoretical point of view, it is interesting to accumulate data as precise as possible on the shape of the dose effect relationships and on RBE dose relationships. Finally, the data obtained by comparing a single fraction and 10 daily fractions of neutrons and gamma rays will add useful information in distinguishing reparable and non reparable lesions.

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Titre du projet n° 4 : Studies on the factors influencing the chromosome aberrations in mammals.

Chef du projet et collaborateurs scientifiques :
A. Léonard, G. Decat, Gh. Deknudt, L. Fabry.

In order to estimate genetic risks in man, it is usually necessary to extrapolate animal data. The only system for which such comparative genetic data including those on man can be obtained readily are cultured lymphocytes. If the factors determining the relative radiosensitivity in different species were understood, a better evaluation of the genetic risk for other types of human cells might be made. Such factors related to the genetic structure could be: The total amount of DNA, its density in the nucleus (ratio DNA content to nuclear volume), the number or chromosomes or chromosome arms which may undergo breaks or exchange reactions, the percentage of heterochromatin, the activity and accuracy of repair enzymes etc... . It must be added that intraspecific variability can also arise from methodological factors. The present project had the aim to study the influence of the methodology and nuclear characteristics by comparing the yield of chromosome aberrations induced by ionizing radiations in the lymphocytes of different mammalian species using the BUdR labelling technique.

A preliminary study using the conventional staining methods was made on seven species, man, chimpanzee, rabbit, pig, sheep, goat and cow. Man, chimpanzee and rabbits have nearly the same number of chromosomes (46,48 and 44) and of chromosome arms but vary greatly with respect to the relative nuclear volume as estimated from the area covered by the lymphocyte nuclei whereas the other four species have different numbers of chromosomes (38, 54, 60 and 60 respectively) but a similar nuclear DNA content, and equivalent nuclear volume and almost the same chromosome arm number ($\pm$ 60). The results obtained with a standardized culture time of 48 hours demonstrated that the radiosensitivity of lymphocyte chromosomes from different

mammalian species is apparently not influenced by characteristics such as nuclear volume, DNA content, chromosome number or number of arms.

In order to determine the existence of some difference in the response to mitogen stimulation among the mammalian species further experiments were made using the 5-bromodeoxy-uridine-Giemsa technique to distinguish the cells in the first mitosis. When the observations are made on cells in second or third division after induction some of the unstable aberrations may, indeed, already have been eliminated. The results obtained with this technique showed that 48 h, the standardized time of cultivation used in the studies reported in the literature 97% of the dividing human lymphocytes are still in first division. The situation appeared very different for rabbit since at that fixation time 27% of rabbit lymphocytes are in first division whereas 45% are in second and 28% in third. Providing only cells in first division are examined, there exists, in fact, no significant difference between man and rabbit with respect to the yield of radiation-induced dicentric chromosomes. It appears therefore premature to decide actually for the arm number hypothesis or for any of the others mentioned previously.

Since certain authors suggested that cell kinetics as well as radiosensitivity may vary among different subpopulations of stimulated lymphocytes comparisons were made for these parameters with two different mitogens, phytohaemagglutinin and concanavalin A. The results obtained showed that the lymphocytes stimulated by these two mitogens have nearly the same cell cycle duration and a similar radiosensitivity.

In conclusion, the results obtained on different species and/or with different mitogens suggest that:

1. Between the mammalian species there could exist no difference in the radiosensitivity of lymphocytes providing cells are examined in first mitosis;
2. The different subpopulations of lymphocytes display the same radiosensitivity to chromosome aberration induction.

If confirmed for other species, the extrapolation of experimental results on mammals to man would be extremely easy and could facilitate the estimation of cytogenetic hazards of exposure to ionizing radiation.

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Titre du projet n° 5 : Studies on the interactions between ionizing radiations and chemicals in producing chromosome ab aberrations in mammals.

Chef du projet et collaborateurs scientifiques :
A. Léonard, Gh. Dekundt and E.D. Léonard.

Numerous mutagenic compounds have been tested for their ability to produce transmissible chromosome aberrations in spermatogonial germ cells and gave, generally, negative results. It is noteworthy that most of the chemicals that failed to induce reciprocal translocations in spermatogonia have been shown to be potent mutagens in other organisms or other test systems and are even capable of causing chromosome breakage in the post-meiotic male germ cells of the mouse as demonstrated by the dominant lethality test. Furthermore, some compounds are able to produce chromosome breaks resulting in the killing of spermatogonia. In the present experiment several chemical substances have been tested alone or in combination with ionizing radiations in different systems (somatic and/or germ cells) to study the interaction between physical and chemical mutagens in the production of chromosome aberrations compatible with the survival and proliferation of mammalian germ cells.

Confirming the data reported with other chemicals, our first experiments with additional chemicals such as hycanthone, styrene oxide, griseofulvin, etc... gave negative results in spermatogonia. A series of experiments was therefore performed with mitomycin C, thio-TEPA and Endoxan. Mitomycin C which fails to produce chromosome rearrangements in premeiotic male germ cells is, however, able to produce chromosome breaks resulting in the killing of spermatogonia as shown by the induction of a temporary sterile period. Endoxan appears less active on spermatogonial cells whereas thio-TEPA is one of the few chemicals inducing a low yield of reciprocal translocation in spermatogonia. The three chemicals were given at different intervals of time (1/2h, 1h, 2h, 6h, or 24h) before or after the exposure to a dose of 400 rad of ionizing radiation (X-rays). Such X-ray dose is able to induce 8 to 9% of spermatogonia carrying chromosome aberrations. The animals were killed 3 to 5 months after treatment.

Pre- or post-treatment by the chemicals resulted, generally, in a decrease of the yield of cells bearing chromsome aberrations.

Injection of 3 mg/kg Mitomycin C, before or after exposure to 400 R, reduced considerably the yield of abnormal cells. In that respect there was no evident difference between the groups treated before and after 400 R. There is, however, some tendency to an interval-effect relationship in the animals given Mitomycin prior irradiation, the lowest yield of abnormal spermatocytes being observed for the shortest time intervals between i.p. injection and radiation exposure. It is probable that the decrease in the yield of translocated cells is due to a selective elimination of the damaged cells.

Injection with 2.5 mg/kg of thio-TEPA before or after a dose of 400 R X-irradiation diminishes the yield of spermatocytes I bearing chromosome rearrangements. Since thio-TEPA has also powerful clastogenic properties in spermatogonia this effect is probably to be explained too, by a selection against the cells with aberrations.

Similar decrease in the yield of translocated cells was also observed when Endoxan was given after 400 R or 6 h or 24 h before irradiation. In contrast with these results, however, no evident interaction between Endoxan and X-ray-treatment was found when the 400 R were given 1/2 h, 1h, or 2h after Endoxan.

In conclusion, our results demonstrate that chemical mutagens can interfere with ionizing radiation by enhancing the selective elimination of damaged cells. Some comparison between the observations made with thio-TEPA and the data reported in the literature with TEM suggests, however, that a pretreatment with this compound could act as a conditioning small dose of ionizing radiation because the results obtained with both compounds are very similar to those obtained in fractionation experiments with X-rays. Furthermore our findings confirm that spermatogonia constitute a relatively safe stage with respect to the possibility of inducing heritable chromosome aberrations by chemical mutagens.

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CONTRACT No.: 218-76-1 BIO D

HEAD OF RESEARCH TEAM: Prof. Dr. W. Gössner

GENERAL SUBJECT OF CONTRACT:

Pathogenesis of radiation damage

The research work carried out during 1976 - 1980 has continued on long-term studies of the late effects of the short-lived alpha-emitting bone-seekers ^{224}Radium and ^{227}Thorium. These studies have been extended to short-lived beta-emitting bone-seekers, especially ^{177}Lutetium. This work was initiated in order to investigate the oncogenic effect of short-lived nuclides in dependence on the skeletal dose and their spatial and temporal distribution.

It has been shown that after single injection of ^{224}Radium in varying amounts (from 37 kBq [1μCi)/kg to 1.85 MBq [50μCi]/kg) the osteosarcoma incidence ranges from 7% to 20% without clear mathematical function on dose dependence. It has been of considerable interest that repeated application of ^{224}Radium (11 Gy [1100 rad] over 36 weeks) increased bone tumor incidence in the longest protraction period and at low dose rate to nearly 100% and shortened the latency period. A similar protraction effect has been demonstrated in experiments with repeated application of the short-lived beta-emitter ^{177}Lutetium. In addition experiments with simultaneous exposure to the short-lived alpha-emitter ^{227}Thorium (half-life 18.7 days) and a low amount of its mother-nuclide ^{227}Actinium (22 years half-life) have shown a more than additive effect with regard to osteosarcoma risk.

As a practical consequence for radiation protection it should be stressed that short-lived radionuclides - in particular with multiple incorporations - may be equally or even more hazardous than a single incorporation of a long-lived radionuclide. This consequence turns out to be true in certain dose ranges for alpha- as well as for beta-emitters.

In addition these studies have been devoted to the question to what extend the risk of radiation-induced bone tumor will be modified by various endogenous factors like age of the animal or strain susceptibility. Studies on the influence of age in radiation-induced osteosarcoma induction demonstrated that young animals (1 month old) during the time of rapid skeletal growth were not at higher risk than adults (6 months old). With regard to strain susceptibility (influence genetic background) it has been shown that a mouse strain with a high incidence of spontaneous osteomas has not a higher osteosarcoma risk than other strains investigated. On the other hand some of the mouse strains studied showed differences in the osteosarcoma latency period.

These experimental studies on short-lived radionuclides appear to be relevant to problems of radiation hazard in man since the occurrence of osteosarcoma has been reported in humans after treatment with ^{224}Radium and this nuclide is still used in the treatment of ankylosing spondylitis. Therefore in addition to the experimental work epidemiological studies on patients treated with ^{224}Radium have been continued. The incidence of malignant tumors the occurrence of exostosis, liver and kidney disease and cataracts has been examined. The follow-up of a rather large group of patients with ankylosing spondylitis treated in 15 orthopaedic and rheumatic hospitals in Western Germany including a control group without radiation treatment has also been continued. These epidemiological studies may also be relevant for the general risk estimation concerning late effects after internal irradiation in man.

PROJECT 1

W. Gössner, U. Linzner, A. Luz, W.A. Müller, E.H. Schäffer

Late effects after incorporation of bone-seeking radionuclides

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1. Bone tumor risk after incorporation of short-lived α-emitters in mice

a) Influence of time factor on bone tumor induction after incorporation of ^{224}Radium

In this study the term "protracted internal irradiation" will be used in case of repeated injections of the radionuclide with time intervals corresponding to the half-life of the nuclide. This will result in a more or less continuous irradiation. The term "fractionated internal irradiation" will be used in case of repeated injections with much longer intervals leading to intermediate decrease of the mean skeletal dose rate to nearly zero Gy/day. The increased osteosarcoma yield observed after protracted internal α-irradiation with 10.80 Gy (1080 rad) over 9 months (^{224}Ra injections twice weekly) could not be found at the lower skeletal dose level of 1.2 Gy (120 rad). Fractionated internal α-irradiation during 9 months (monthly injections of ^{224}Ra at the dose level of 10.80 Gy [1080 rad]) resulted in a longer latency period and a lower number of osteosarcoma per mouse as compared to the effect of protracted irradiation.

b) Simultaneous incorporation of ^{227}Thorium and its long-lived mother-nuclide ^{227}Actinium

The final osteosarcoma induction after simultaneous incorporation of 1.85 kBq (0.05 μCi)/kg ^{227}Ac and 185 kBq (5 μCi)/kg ^{227}Th is not significantly different from an additive effect.

	^{227}Ac : 12%	(6/50)
	^{227}Th : 46%	(23/50)
^{227}Ac + ^{227}Th :	60%	(30/50)

But for the first 700 days of the experiment the osteosarcomagenic effect after incorporation of both nuclides is more than additive.

	^{227}Ac : 8%	(4/50)
	^{227}Th : 38%	(19/50)
^{227}Ac + ^{227}Th :	58%	(29/50)

In a recent experiment, varying the amount of ^{227}Th (18.5 kBq [0.5µCi], 74 kBq [2.0µCi], 185 kBq [5.0µCi]/kg), the osteosarcomagenic effect 553 days after simultaneous incorporation of 1.85 kBq (0.05µCi)/kg ^{227}Ac and ^{227}Th was also more than additive and significantly increased as compared to incorporation of ^{227}Th alone.

c) The influence of age on the bone tumor induction by short-lived α-emitters

Incorporation of ^{227}Th beyond the period of rapid skeletal growth reduced the total mean skeletal dose by a factor of 0.7 as compared to rapidly growing mice at 4 weeks of age. The osteosarcoma risk after single or fractionated incorporation of 185 kBq (5µCi)/kg ^{227}Th (expectancy of osteosarcoma incidence 40% and 80%) in 6 months old mice was not significantly reduced as compared to 1 month old animals. The tumor latency period for the older animals was shorter especially in the group with lower tumor expectancy, where a relation to the time of appearance of spontaneous osteosarcomas seems to exist.
Incorporation of 185 kBq (5µCi)/kg ^{227}Th in 18 months old mice (the highest age which was investigated) did not result in any osteosarcoma case during the rest of the life span.

d) Incorporation of short-lived α-emitters in newborns and during pregnancy

The mean skeletal dose after incorporation of ^{227}Th in newborns as compared to 1 month old mice is reduced by a factor of 0.4. Long-term experiments after incorporation of 3.7 kBq (0.1μCi)/kg and 37 kBq (1μCi)/kg showed no higher sensitivity for bone tumor development as compared to 1 month old mice. Incorporation of 5 x 18.5 kBq (0.5μCi) per kg ^{224}Ra during pregnancy resulted in maximal 0.05 Gy (5 rad) mean skeletal dose for the offspring. As expected from a linear dose dependence in juvenile mice the final osteosarcoma incidence was not significantly different from untreated control animals (maximal 2%).

e) Influence of strain (genetic background) on the osteosarcoma induction by 185 kBq (5μCi)/kg ^{227} Thorium in female mice

The following strains have been investigated in comparison to NMRI mice: (C3H x 101)F_1, 101, BALB/c, C57Bl, X/Gf, AKR. The osteosarcomagenic effect in NMRI (outbred, high incidence of spontaneous lymphoma) and (C3H x 101)F_1 mice (inbred, low incidence of spontaneous lymphoma), 101 mice (inbred, 70% spontaneous osteoma) was not significantly different. The osteosarcoma latency period tested by the logrank test was different with the following sequence of strains: BALB/c, C57Bl, NMRI (Figure 1). The higher sensitivity of BALB/c as compared to NMRI is significant with regard to this parameter.
The - according to literature - low tumor prone X/Gf mice show 553 days after incorporation no significant lower sensitivity than the NMRI.
During the naturally short life-span of the lymphoma prone AKR mice no significant osteosarcoma induction by incorporation of ^{227}Th could be observed. Therefore this strain does not show a peculiar sensitivity for bone tumor induction regardless of its background of endogenous retroviruses.

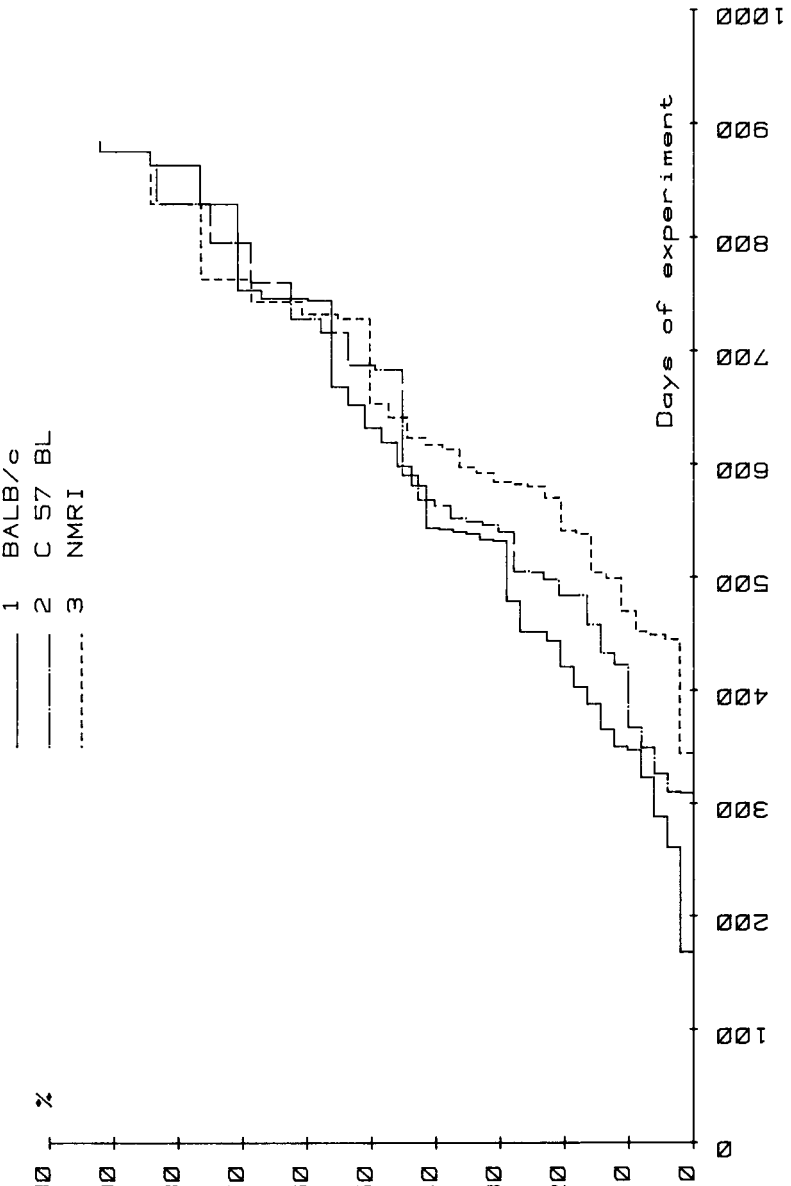

FIGURE 1
Comparison of the tumor induction in BALB/c, C57Bl and NMRI mice. Osteosarcoma incidence corrected for competing risks according to Miescher et al. Groups of 50 female mice, 4 weeks of age received 185 kBq (5µCi)/kg ^{227}Th.

2. Bone tumor risk after incorporation of short-lived β-emitters in mice

Long-term studies with short-lived β-emitters were carried out analogous to that with α-emitters. These studies should also give an insight into the problems of Relative Biological Effectiveness (RBE).
Several radionuclides of the rare earths have been investigated and incorporation studies have been performed with ^{141}Ce, ^{153}Sm, ^{165}Dy, ^{175}Yb, and ^{177}Lu. The retention in the different organs was influenced by the amount of the stable element. The highest skeletal dose was reached with a minimum of stable carrier.
Long-term studies were then concentrated to ^{153}Sm and ^{177}Lu. Most long-term experiments for osteosarcoma induction were performed with ^{177}Lu (half-life 6.7 days, β-Max.-Energy 0.5 MeV) with a concomitant carrier of 0.025 mg/kg. The mean skeletal dose was 5.6 Gy (560 rad) per 1 mCi/kg ^{177}Lu (37 MBq/kg) i.p. injected. Single injections were applied as well as repeated injections of small fractions of activity over 12 weeks with one weekly injection.
Figure 2 shows the cumulative incidence of osteosarcomas in female NMRI mice with three different doses (1-3 single injections, 4-6 repeated injections of small fractions). As illustrated in Figure 2 and listed in Table 1, with 370 MBq (10mCi)/kg and 740 MBq (20mCi)/kg the final incidence of osteosarcomas was doubled by protraction (from 37% to 80%). With 185 MBq (5mCi)/kg this protraction effect was lower (12.5% to 20%) and not significant. The latency times were shortened both by the increase of dose and by protraction of the irradiation period.
Table 1 shows in addition the tumor incidence per Gy (column 7). Here we found the highest value for a mean skeletal dose of 56 Gy protracted over 12 weeks. Compared to our results with α-doses these values are about one tenth, consequently suggesting a RBE value of 10 for α-emitting bone-seekers in agreement with the data of the literature.

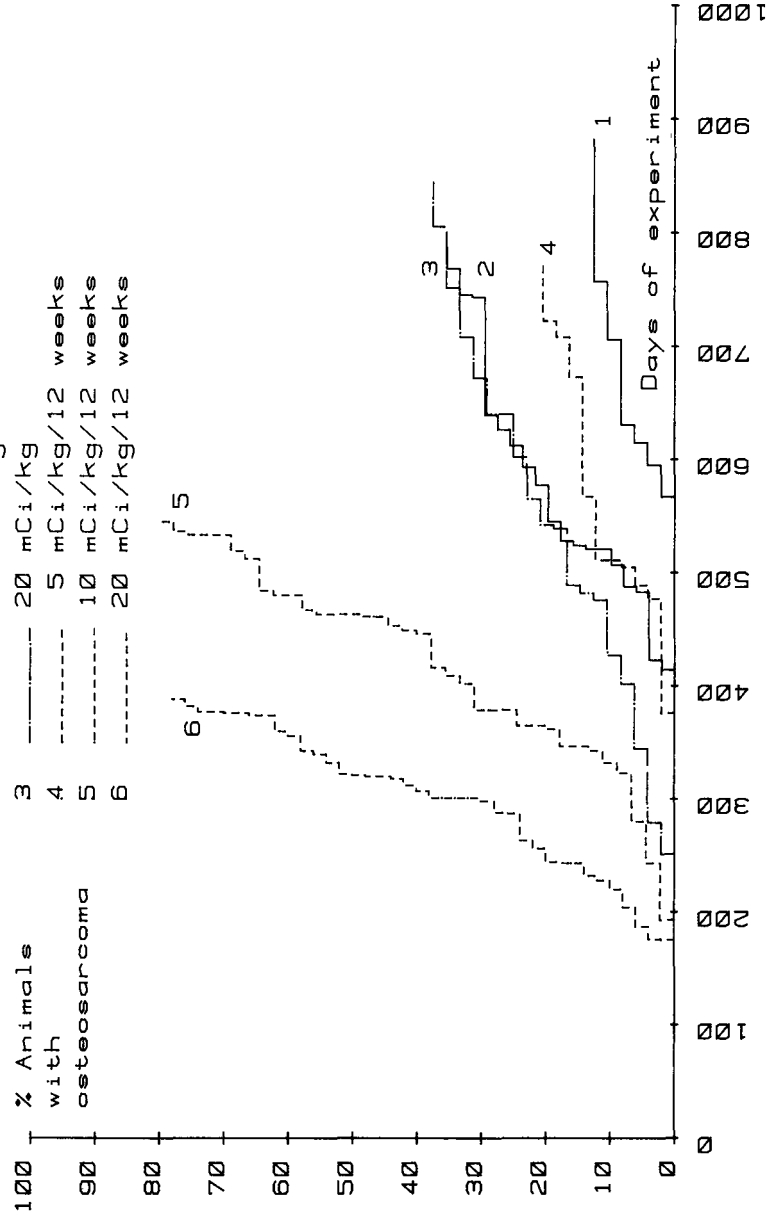

FIGURE 2

Cumulative osteosarcoma incidence with three different skeletal doses after one single incorporation of ^{177}Lu or repeated incorporation of small fractions with time interval of the half-life (i.e. protracted internal irradiation).

TABLE 1 Osteosarcoma incidence in NMRI mice after injection of beta-emitting radionuclides

Radionuclide	Total injected activity	Mean skeletal dose Gy 1 Gy =100 rad	Max. mean skeletal dose rate Gy/d	Application: a) single b) 12 weeks protraction	Final bone tumor incidence %	Bone tumor incidence per Gy %	Mean latency period (days)
Lu-177	5 mCi/kg 185 MBq	28	3.2	a)	12.5 (6/48)	0.45	645
Lu-177	5 mCi/kg 185 MBq	28	0.36	b)	20 (10/50)	0.71	554
Lu-177	10 mCi/kg 370 MBq	56	6.4	a)	35.3 (18/51)	0.63	570
Lu-177	10 mCi/kg 370 MBq	56	0.72	b)	80.0 (40/50)	1.43	421
Lu-177	20 mCi/kg 740 MBq	112	12.8	a)	37.5 (18/48)	0.33	534
Lu-177	20 mCi/kg 740 MBq	112	1.44	b)	78.0 (39/50)	0.70	301
Sr-90	100 µCi/kg 3.7 MBq	28	0.19	a)	24 (12/50)	0.87	753
Sr-90	500 µCi/kg 18.5 MBq	80	0.93	a)	70 (35/50)	0.88	347

A reference experiment with two doses of the long-lived β-emitter ^{90}Strontium (single injected) was carried out and the results are added at the end of Table 1:
As shown with the 28 Gy skeletal dose of ^{90}Sr, the osteosarcomagenic effect expressed as final incidence percentage is comparable to that of the same skeletal dose with protracted ^{177}Lu, but, is lower per Gy (0.87%) compared to the effect of protracted ^{177}Lu dose of 56 Gy (1.43%). The mean latency period for the 28 Gy ^{90}Sr is remarkably longer (753 days) than that of Lu-protraction of the same skeletal dose (554 days). This could be due to the lower skeletal dose rate (column 4) after ^{90}Sr incorporation compared to the ^{177}Lu case.

PROJECT 2

V. Erfle, W. Gössner, A. Luz, K.-H. Marquart, A.B. Murray, E.H. Schäffer

Pathogenesis of early and late effects after internal irradiation

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1. C-type retrovirus expression

In the past five years period our efforts in project 2 have been concentrated on the role of endogenous C-type retroviruses in radiation-induced osteosarcomagenesis.
There were three points of major interest:

- the presence of C-type viruses or viral structural components in the osteosarcomas;
- the appearance of C-type viruses during the course of the latency period and
- the evaluation of the properties of C-type viruses isolated from the osteosarcomas.

a) Virus particles were observed by electron microscopy in ^{224}Ra and ^{227}Th-induced osteosarcomas from both (C3H x 101)F_1 and NMRI mice. Osteosarcomas from NMRI mice, however, contained mainly intracisternal A-type virus particles and no C-type particles whereas osteosarcomas from (C3H x 101)F_1 mice contained varying quantities of mainly C-type particles and a few A-type particles.
These results could be confirmed by the detection of viral particles in primary and transplanted osteosarcomas with the typical properties of C-type retroviruses as density of 1.16 g/cm^3 in sucrose, a 60-70 S RNA and a RNA dependant DNA polymerase. Further evidence of the association of these tumors with retroviruses was the content of viral p30 protein. Primary sarcomas of (C3H x 101)F_1 and NMRI mice had mean p30 values of about 110-130 µg p30/g tissue, transplanted tumors up to 1000 µg p30/g tissue. Interestingly viral p30 protein was also elevated in spleen, kidney and serum of

tumor mice (about 160 or about 190 µg/g tissue resp. and about 0.8 µg/ml serum) in contrast to muscle and bone tissue (about 0.1 or about 0.3 µg/g tissue). The p30 protein in the tumors could be localized by the immunperoxidase staining technique in the cytoplasm of the sarcoma cells. The radiation-induced osteosarcomas could not be transmitted by cell-free extracts. But "in vitro" cultivation of the tumors led to the production of typical murine C-type retroviruses with oncogenic properties (see c).

b) The search for C-type viral expression during the latency period was tested in (C3H x 101)F_1 mice with ^{224}Ra treatment (0.5 µCi/kg twice weekly over 36 weeks) resulting in an osteosarcoma incidence of nearly 100%. Early transient activation of endogenous C-type viruses appeared in the bone tissues in the first month after start of irradiation. This was followed by a marked production of viral antibodies. The antibodies were directed against ecotropic murine C-type viruses (replicating in mouse cells) and reacted exclusively with the glycoprotein gp70 of the virus envelope. The viral antibodies remained elevated in the latency period until the time of tumor appearance (month 8-12 after start of irradiation). This time is characterized by a second appearance of retrovirus expression in bone tissues, a steep decrease of the viral antibodies and a transient increased content of viral p30 protein in the serum. Tumor animals again exhibited viral expression in the osteosarcomas, increased p30 content in serum, and a lack of viral antibodies.
Parallel electron microscopic studies showed the presence of varying amounts of both mainly immature C-type virus-like particles and atypical pleomorphic mature C-type particles at all stages after irradiation as well as in untreated controls. Typical mature C-type particles were not found.

c) The viruses isolated from "in vitro" cultivated radionuclide-induced sarcomas showed the major properties of murine C-type retroviruses: C-type morphology, density in sucrose 1.16-1.17 g/cm^3, 60-70 S RNA with poly A, murine reverse

transcriptase (Mn ≫ Mg), and the major structural proteins (p10, p12, p15, p15E, p30, gp70). Serological analysis revealed a group-specific p30 protein of murine viruses, a type-specific p12 protein and gp70 glycoprotein of N-tropic murine viruses with some crossreactivity in p12 and gp70 to the xenotropic murine viruses. Biologically the viruses behaved as N-tropic ecotropic murine C-type viruses. After inoculation in syngeneic newborn mice, two viral isolates from radionuclide-induced osteosarcomas induced either fibrosarcomas in C3H mice or lymphomas and osteomas in NMRI mice, but never osteosarcomas.

The present results show a clear association of endogenous C-type retroviruses with the development of radiation-induced osteosarcomas by the following events:

- the activation of viruses by internal irradiation;
- the appearance of viruses during tumor development and in the tumors;
- the creation of oncogenic viruses during the sarcomagenic process.

Concerning the pathogenesis of the radiation-induced osteosarcomas a process can be postulated which in principle could be analogous to the development of C-type sarcoma viruses. The radiation activated viruses may recombine with normal cellular gene material which is then integrated by the infectious virus in the cells at a site of the genome devoid of appropriate repressor functions and thus leading to uncontrolled growth. On the other side viral expression is significantly changed after irradiation and during tumor development. Thus viral parameters could serve as markers for exposure to internal α-irradiation and for the development of sarcomas in mice of different strains.

2. Preneoplastic lesions and early stages of tumor development

Non-neoplastic foci of cellular proliferation can be found in the skeleton of otherwise healthy female old mice in all strains studied until today (NMRI, C3H x 101/F_1, 101, BALB/c, C57Bl). The extension and intensity of this lesion did not

show any relation to differences of sensitivity in bone tumor induction by internal irradiation (see project 1). A detailed study of the epiphyseal-metaphyseal region of the long bones was performed in (C3H x 101)F_1 mice in an experiment with high osteosarcoma expectancy (2 x 0.5 µCi/kg ^{224}Ra per week, total activity 36 µCi/kg). In males induction of non-neoplastic proliferations and in females higher rate of occurrence of these proliferations - morphologically similar to the spontaneous lesions - has been observed. The frequency of non-neoplastic proliferations was much higher than the frequency of small intraosseous and gross osteosarcomas. The increased occurrence of the non-neoplastic lesions has been observed only shortly before the observation of neoplasms.

PROJECT 3

W. Gössner, A. Luz, W.A. Müller, E.H. Schäffer

Combined effects

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1. Internal irradiation and stimulation of osteogenic tissue

a) Application of Fluoride during the latency period of ^{227}Thorium-induced osteosarcoma

Female Wistar rats received 70 ppm Sodium Fluoride in the drinking water for 52 weeks, starting about 13 weeks after incorporation of 185 kBq (5µCi)/kg ^{227}Th. There was no significant influence of this treatment on the development of radiation-induced osteosarcoma. The osteosarcoma incidence in the different groups was:

controls	0/40
Fluoride	0/40
^{227}Th	63% (24/38)
^{227}Th + Fluoride	71% (27/38)

b) Promotion of ^{227}Thorium-induced osteosarcoma by lathyrogenic diet

β-Aminopropionitrile-fumarate (BAPN) has been applied at a dose level which induces exostosis-like local bone hyperplasia. Female Wistar rats received 500 mg BAPN per 100 g food during 5 time period (days 242-262, 284-304, 372-392, 423-444, 470-490) after incorporation of 185 kBq (5µCi)/kg ^{227}Th. By this treatment the development of osteosarcomas could be accelerated (incidence 9/15 instead of 1/15, 400 days after incorporation), but the finally achieved tumor incidence was identical in the groups with and without BAPN treatment (12/14 = 86%). No osteosarcoma could be induced at the site of BAPN-induced bone hyperplasia. Therefore the mechanism of tumor promotion by BAPN is not clear.

2. Stimulation of the immune system during the early latency period of ^{227}Thorium-induced osteosarcoma

Female NMRI mice received 8 injections of 20µg Lipopolysaccharide (LPS, E.coli 0111:B4) with time interval 3.5 days starting 4 weeks after incorporation of 185 kBq (5µCi)/kg ^{227}Th. This stimulation of the B-cell system and activation of endogenous viruses (as described in the literature) did not significantly influence the osteosarcoma development. The osteosarcoma incidence was not different in both groups.

3. Administration of drugs interfering with immune reactions and possible translation of virogenes during the osteosarcoma latency period

a) Treatment with Cyclophosphamide

Monthly injections of 60 mg/kg Cyclophosphamide during month 3 to 10 after incorporation of 185 kBq (5µCi)/kg ^{227}Th resulted in a significantly (logrank test) shorter latency period of radiation-induced osteosarcoma in female NMRI mice (see Figure 3).
Treatment with two injections of 150 mg/kg Cyclophosphamide 1 day and 4 weeks after incorporation of 185 kBq (5µCi)/kg ^{227}Th in female BALB/c mice increases slightly, but significantly ($p < 0.05$) the osteosarcoma incidence 583 days after incorporation. The osteosarcoma incidence was 36% (27/74) with ^{227}Th alone and 20% (15/74) after additional treatment with Cyclophosphamide.

b) Treatment with Daunomycin

442 days after incorporation of 185 kBq (5µCi)/kg ^{227}Th in female BALB/c mice the osteosarcoma incidence was 10% (5/50) and 6 weekly injections of 1 mg/kg Daunomycin, starting 1 day after incorporation of the ^{227}Th, did not influence this result.

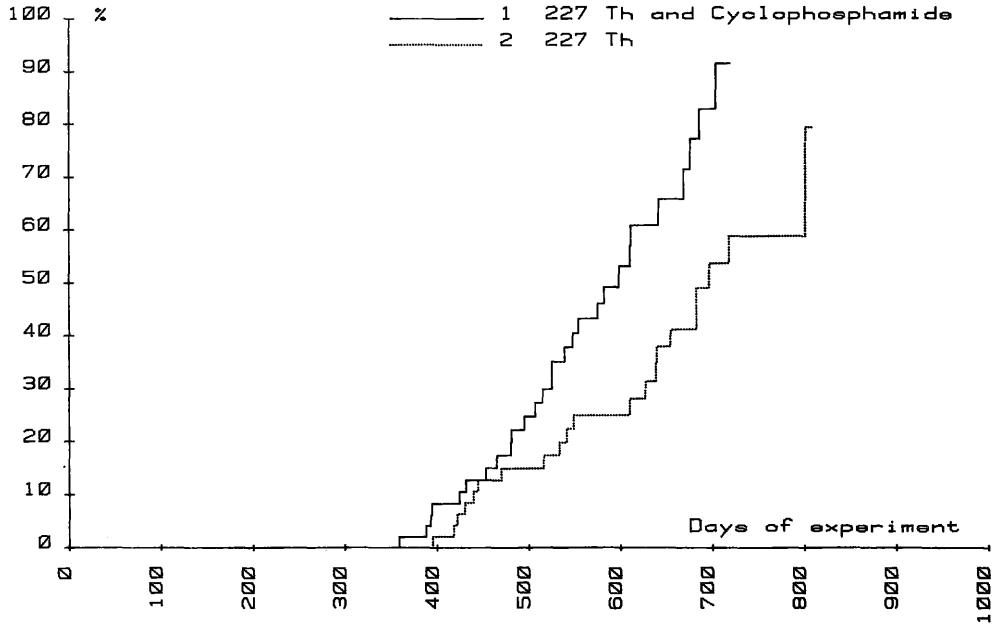

FIGURE 3

Osteosarcoma incidence corrected for competing risks according to Miescher et al. Groups of 50 female NMRI mice, 4 weeks of age.

Group 1 i.p. injection of 185 kBq (5µCi)/kg ^{227}Th plus 7 monthly injections of 60 mg/kg Cyclophosphamide during month 3 to 10 of the experiment

Group 2 i.p. injection of 185 kBq (5µCi)/kg ^{227}Th only

PROJECT 4

Epidemiological studies on late effects in ^{224}Ra-treated patients

Study A (H. Spiess)

Nine-hundred patients (682 adults and 218 juveniles) have been followed up about 20 years since repeated injections (average of 666 kBq [18μCi]/kg ^{224}Ra). ^{224}Ra was applied for the treatment of tuberculosis, ankylosing spondylitis, and other diseases (Tables 2 and 3). ^{224}Ra concentrates mainly in bone, but an appreciable fraction of ^{224}Ra and its daughters decay in soft tissues. Bone sarcomas have been the most dramatic skeletal effect from the high-dose treatment. In addition benign exostoses, growth retardation, teeth breaking, and cataracts have been observed.

There is a high number of kidney and liver diseases (mostly cirrhosis). The pathogenesis of this lesion in relation to Peteosthor and pure ^{224}Ra is at present an open question.

TABLE 2 Follow-up study of ^{224}Ra-treated patients - December 1980

Total number: 1810
not found: 242, physicians report without name: 654,
investigation refused: 16, exactly controlled: 900

Age at beginning of ^{224}Ra treatment	1-20 years	after 21 years	total number
reply received	218	682	900
report by physician or questionnaire	49	326	375
personally contacted or hospital examination	107	108	215
deceased	77	335	412
cause of death			
original disease	11	42	53
not in relation to original disease	13	145	158
unknown	6	38	44
malignant bone tumors			
osteosarcoma	30 (+ 2L)	16 (+ 2L)	46 (+ 4L)
chondrosarcoma	4	0	4
other malignant tumors (carcinoma)	6 (+ 2L)	35 (+12L)	41 (+14L)
diseases of the liver	1	19	20
diseases of the kidney	5	35	40
panmyelophthisis, anaemia	1	3	4
leukemia	0	3	3
other lesions			
benign bone tumors			
osteochondroma solitary	7	0	7
multiple	20	0	20
osteochondroblastoma	1	0	1
osteofibroma	1	0	1
early broken teeth	23	12	35
growth retardation	50	0	50
cataract	11	26	37

L = living patients

TABLE 3 Changes between 1976 and 1980

Age at beginning of ^{224}Ra treatment	1-20 years	after 21 years	total number
death during the last 4 years	3	44	47
cause of death			
bone sarcoma cases	0	0	0
tumors of soft tissues	1 (+ 1L)	6	7 (+ 1 L)
leukemia	0	1	1
panmyelophthisis, anaemia	0	1	1
diseases of the liver	0	4	4
diseases of the kidney	1	4	5
exostoses	0	0	0
early broken teeth	4	1	5
cataract	0	4	4

Study B (W. Gössner, R.R. Wick)

This study (Table 4) on late effects in man after incorporation of ^{224}Ra in the low level range (less than 237 kBq [6.4µCi]/kg ≙ less than 0.9 Gy [90 rad] α-skeletal dose) was considerably enlarged and it was supplemented by a control group of patients never treated either with X-rays or radionuclides.

At the end of 1980 the study involves 1543 patients from 14 hospitals of the FRG and 706 not exposed control patients. 840 respectively 264 could be controlled exactly; 297 respectively 127 have died.

Up to now, 2 cases of tumors of the skeleton have occurred at a skeletal burden less than 0.9 Gy [90 rad]. One was a reticulum cell sarcoma of the vertebral column, the other a fibrosarcoma at the ileo-sacral joint. The observation of these 2 cases of relatively rare tumors of the skeleton show, that even in the low dose range, radiation-induced tumor risk in the skeleton cannot be ruled out.

With regard to liver and kidney diseases the results of the study A could not be confirmed until today. In this connection it might be interesting to consider that most of the patients of the Spiess group have been treated with Peteosthor (mixture of ^{224}Ra with eosin and colloidal platinum), while the majority of patients of this study have been injected with pure ^{224}Ra.

TABLE 4 Death causes of patients

death causes	exposure group mean age at death = 59,1 a number of patients	%	control group mean age at death = 61.0 a number of patients	%
Tuberculosis	7	2,3	3	2,4
Neoplasms	39	13,1	23	18,1
(Neoplasms of Digestive Organs)	(19)	(6,4)	(10)	(7,9)
(Neoplasms of Stomach)	(5)	(1,7)	(4)	(3,2)
Heart & Circulatory Diseases	105	35,3	51	40,1
Respiratory System	21	7,1	6	4,7
Digestive System	13	4,4	15	11,8
(Liver Diseases)	(6)	(2,0)	(11)	(8,7)
Urinary System	18	6,1	5	3,9
(Kidney Diseases)	(18)	(6,1)	(5)	(3,9)
Nervous System	23	7,7	10	7,9
(Apoplexy)	(22)	(7,4)	(10)	(7,9)
Other Diseases	37	12,5	4	3,2
Suicides	8	2,7	4	3,2
Accidents, Violence	26	8,8	6	4,7
TOTAL	297	100,0	127	100,0

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A. Contractual Partner of the Commission:

Prof.Dr. K.E. Scheer: Director of the Institute of Nuclear Medicine, German Cancer Research Center Heidelberg

Contract No.: 100-76-1 PST D

Head of the Research Group: Prof.Dr. K.E. Scheer, Director of the Institute of Nuclear Medicine, German Cancer Research Center Heidelberg

Assistant Head of the Research Group: Prof.Dr. W.J. Lorenz, Institute of Nuclear Medicine, German Cancer Research Center Heidelberg

Coordinator: Prof.Dr. G. van Kaick, Institute of Nuclear Medicine, German Cancer Research Center Heidelberg

The contracted research program is to be carried out at the Institute of Nuclear Medicine, German Cancer Research Center Heidelberg, in collaboration with:
Prof.Dr. H. Muth, Director of the Institute of Biophysics, University of Saarland (Boris-Rajewski-Institut) Homburg

Prof.Dr. G. Wagner, Director of the Institute of Documentation, Information and Statistics, German Cancer Research Center Heidelberg and

Prof.Dr. A. Kaul, Department of Nuclear Medicine, Free University of Berlin

General Topic of the Contract:

Research Project "Thorotrast" - Investigations to Evaluate the Long Term Effects Caused by Artificial Radiation in Man (Thorotrast-Patients) - Follow up - Study.

B. Project Title:

a) Clinical, Radiological and Biophysical Examinations of Thorotrast- and Control Patients

b) Investigating the Final Fate of Deceased Patients of Both Groups

Head of the Project: Prof.Dr.K.E.Scheer (Contractual Partner)
Prof.Dr.W.J.Lorenz (Assistant Head of Project)
Prof.Dr.G. van Kaick (Coordinator)

Scientific Collaborators of the Institute of Nuclear Medicine:
Dr.H.Lührs, Dr.H.A.Müller, Dr.L.Strauß

Statistical Evaluation: Prof.Dr.G.Wagner, Prof.Dr.H.Immich, Dr.H.Wesch

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a) During 1976-1980 1164 examinations of 751 patients (378 thorotrast patients and 373 control patients) were performed. They included a clinical status, biochemical, immunological, hematological tests, radiological findings, and biophysical measurements. Special attention was given to the liver by examination with echography, radionuclide imaging and computerized tomography. Many thorotrast patients suffered from diffuse parenchymal diseases and often from primary liver tumors. The combination of the three imaging methods means real advantage in liver diagnostics. The evaluation of the large amount of clinical data and findings cannot be presented here. It should be mentioned that the analysis of X-ray pictures resulted typical patterns of thorotrastosis correlating to the volume of thorotrast injected intravascularly. The evaluation of the laboratory tests showed significant differences between thorotrast and control groups only with regard to the bromsulfthalein test. These differences did not correlate to dose, dose rate and exposure time. The hematological status of the thorotrast patients did not reveal significant alterations. A recent serological study (not yet published) of 500 blood samples (thorotrast- and control patients) clearly showed that chronic infection with hepatitis-B-virus is not influencing the cancerogenisis as a co-factor.

Thorotrast patients with paravascular deposits often suffered from paralysis of the adjacent nerves or from chronic inflammation. We did not find however any neoplastic lesion. In general the close contact to the family physicians and the hospital doctors was continued and proved to be very helpful for the investigation of the final fate of the patients.

b) Until now 429 thorotrast patients and 122 control patients of the examined group have died (Tab.1). The causes of death of these patients were clarified by intensive correspondence with the family physicians, hospitals, institutes of pathology and health offices. For example, almost all listed liver tumors could be confirmed by histological findings. The high excess rate of liver tumors is the most striking result of the study. Pathoanatomically these tumors were classified as: Cholangiocellular carcinomas (40%), hemangioendothelial sarcomas (35%) and hepatocellular carcinomas (25%). We looked for the effect of the mean α-dose rate in the liver on carcinogenesis. The thorotrast patients were divided in 3 subgroups according to the measured Tl-208 activity (excluding patients with large paravascular deposits):

1) 82 patients, mean dose rate to the liver 14 rad/y
2) 121 patients, mean dose rate 21 rad/y
3) 67 patients, mean dose rate 35 rad/y.

Fig.1 shows the cummulative incidence of liver tumors in the 3 subgroups. It is evident that the increment of the curve depends on the mean dose rate. The same evaluation of data with regard to the myeloid leukemias did not show any correlation between incidence and dose rate to bone marrow!

An unexpected but very important result of the study is the observation, that until now there is no excess rate of bronchogenic carcinomas in the group of thorotrast patients. The radiation burden to the epithelial cells of the bronchi caused by the exhaled thoron seems to be without cancerogenic effect (mean accumulated dose after 30 years about 250 rad).

A typical osteosarcoma in the thorotrast group and an atypical malignant bone tumor in the control group was observed. In total three histologically confirmed bone sarcomas were registered in the German thorotrast study (2 cases in the group of the non-examined patients).

Comparing the frequencies of other neoplastic diseases there are no differences, or the figures of the control group are even higher. Vascular diseases are more often causing death in the control group (Tab.2).

Final evaluation of the German thorotrast study can be done when we have gathered more epidemiological data of the examined group. Today we have under observation 472 thorotrast patients and 547 control patients. We have exact and reliable information on anamnesis, state of health and amount of intravascularly injected thorotrast. Therefore it is essential to continue follow-up examinations of these patients.

It should finally be mentioned that in addition to this epidemiological study animal experiments are carried out to estimate the non-radiation effects of thoriumdioxyd (funded by the Federal Ministry of Internal Affairs).

Tab.3 gives a survey of the complete German thorotrast study. Beside the most important group of examined patients we have to look also at the interesting group of non-examined patients (deceased more than 3 years after injection of thorotrast).

This group of patients will bring information on the radiation induced carcinogenesis during the first 20 years of exposure. A first evaluation of these epidemiological data was performed by van Kaick et al. 1977.

Group of examined patients

General Data	Thorotrast	Control
Total number of patients	901	669
Female patients (%)	25	20
Age at injection (y)	28	28
Interval to 1. exam. (y)	26	26
Mean volume Thorotrast (ml)	22	-
Deceased patients	429	122

Tab.1

Diseases of interest of deceased patients

Diagnosis	Frequency (%)	
	Thorotrast (n=429)	Control (n=122)
Primary liver tumor	33,3	0
Cirrhosis or fibrosis of the liver	36,4	6,6
Acute or chronic myeloid leucemia	2,6	0
Acute or chronic lymphatic leucemia	0	0
Aplastic anemia	2,6	0
Morbus Hodgkin	0,5	0
Bronchogenic carcinoma	2,1	4,9
Bone sarcoma	0,2	0,8
Tumor of the g.i. tract	3,0	6,6
Renal tumors	0,5	0
Carcinoma of the prostate	0	0
Carcinoma of the breast	0,2	0,8
Tumor of the brain	0,7	0
Myocardial infarction	5,1	15,6
Cerebral insult	5,4	9,0

Tab.2

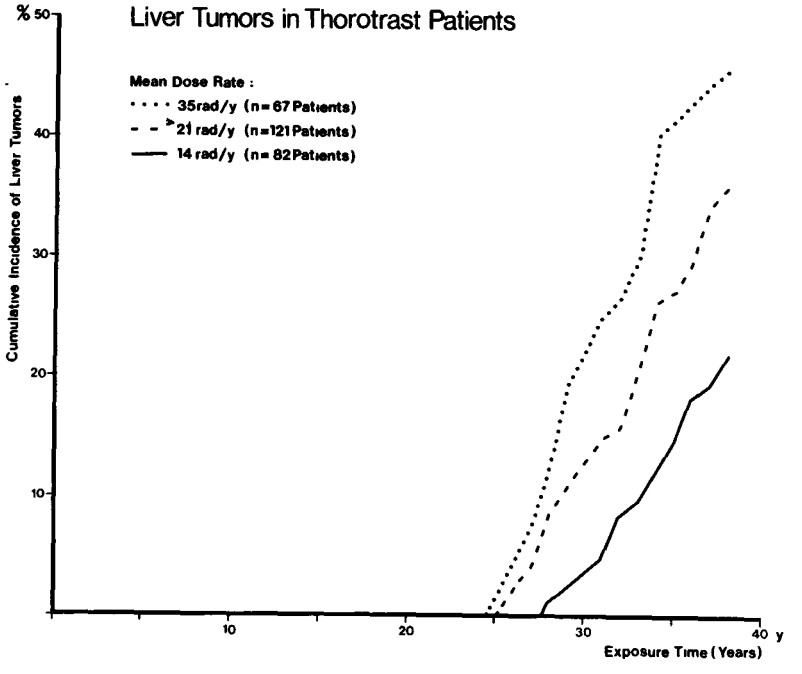

Fig.1

German Thorotrast Study			
Group of Patients	ThO_2	Control	Total
Deceased within 3 y	1918	615	2533
Deceased after 3 y (non-examined)	1257	1168	2425
Examined	901	669	1570
Non-responders	172	1475	1647
Non-traceable	911	1233	2144
Total	5159	5160	10319

Tab.3

List of Publications

Leipolz-Angermüller, S.:
Tierexperimentelle Untersuchungen zur Karzinogenese von Thorotrast in den Organen des RES.
Verhandlungen der Deutschen Gesellschaft für Pathologie, 60.Tagung. Fischer, Stuttgart-New York (1976) 425.

Naser, V.:
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Health Physics 35 (1978) 127-136.

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Late effects and tissue dose in Thorotrast patients.
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Steinsträsser, A.:
Die Strahlenbelastung der Lymphocyten bei Thorotrasttägern.
Ein Beitrag zur Problematik der "Biologischen Dosimetrie" nach Inkorporation von Radionukliden.
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Tierexperimentelle Untersuchungen zur Biokinetik von kolloidalem Zirkoniumdioxyd.
MD.Thesis, Berlin 1979.

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Comparative Investigations on the Biokinetics of Colloidal Thorium, Zirconium, and Hafnium Dioxides in Animals.
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Lührs, H.:
Blutchemische und hämatologische Befunde bei Thorotrastpatienten im Vergleich zu Kontrollpersonen.
Thesis, Med.Fakultät der Univ.Heidelberg, 1979.

Contractor : University of Oxford (Churchill Hospital Research Institute)

Contract nr : 266-78-1 BIO UK

Head of the research team(s) : Dr. J.W. Hopewell and Dr. G. Wiernik

General subject of contract : Measurement of early and late radiation reactions in normal tissues

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Title of the project nr. 1 : The Measurement of Early and Late Effects of Irradiation in Pig Skin

Head of Project and scientific staff : J.W. Hopewell, G. Wiernik, Y. Gunn, J.L. Foster, G. Morris and C.M.A. Young

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The measurements of both the early and late effects of irradiation on the skin have been undertaken in the pig. This animal species has skin structure very similar to that of man and hence any experimental findings can be directly extrapolated to man.

A wide range of complementary assay techniques have been used to examine the initial reaction of pig skin to irradiation. These have included direct visual observations (skin scoring), thermography, dermal blood flow measurements (isotope clearance) and histology.

These observations indicate that in the first 4 months after irradiation there are two distinct waves of response in the skin, which are linked with effects on both epithelium and the vasculature of the dermis. In the first wave reaction, which reaches a peak 4-6 weeks after irradiation, evidence of epithelial cell death and reduced basal cell density can be seen in histological sections, indeed if the dose is high enough desquamation may result. With lower dose levels, an erythema response is observed. Blood flow and skin temperature are increased at this time. Both these changes are seen as an inflamatory reaction to epithelial cell death.

The second wave reaction which occurs 10-16 weeks after irradiation results from radiation damage to the vasculature of the dermis. The skin has a distinct bluish appearance and measurements have shown blood flow and skin temperature to be reduced. Histological evidence of occlusive changes in arterioles, particularly those in the deep dermal plexus and a reduction in vascular density in the dermis, provide direct evidence of vascular damage. High doses (single doses of $\geq$2070 rad) produce severe vascular damage and result in dermal necrosis. With lower doses the effects of vascular damage are transient and blood flow

and skin temperatures gradually return to normal. This is associated with the loss of parenchymal elements from the dermis resulting in late tissue atrophy.

The relative severity of the response of the epithelial and vascular elements in the skin are greatly influenced by dose fractionation. With single doses or a small number of large dose fractions dermal necrosis may result after an interval of 11-16 weeks, without prior epithelial desquamation. On the other hand, small daily doses of 205-267 rad over six weeks (total doses 6150-8000 rad) produces a transient epithelial desquamation at approximately the end of irradiation. No subsequent severe vascular reaction is observed, i.e., no necrosis.

The relative radio-sensitisation of epithelial cells produced by protracted irradiation is associated with the re-assortment of basal layer cells into more sensitive phases of the cell cycle, this occurring towards the end of the 6 week period (Table 1). This may result from a shortening of the cycle time and/or an increase in the growth fraction.

Table 1: Evidence for the re-assortment of basal cells within the cell cycle when daily fractionation is extended over 6 weeks

Nuclear density and labelling index (± SE) of basal cells in pig epidermis after daily irradiation for 2, 4 and 6 weeks
Values related to those in normal skin

Treatment Group		Nuclei/mm (%)	Labelling Index (%)
2 Weeks (10 fractions)	(a)	—	—
	(b)	87.85 ± 2.60	42.52 ± 5.79
	(c)	79.61 ± 2.79	31.41 ± 3.69
4 Weeks (20 fractions)	(a)	53.28 ± 3.77	32.53 ± 19.27
	(b)	54.22 ± 1.89	52.15 ± 7.0
	(c)	58.96 ± 3.52	65.36 ± 6.66
6 Weeks (30 fractions)	(a)	35.89 ± 1.89	361.45 ± 48.19
	(b)	61.18 ± 2.71	377.33 ± 31.22
	(c)	54.35 ± 2.39	191.41 ± 16.94
E.O.T. + 2 weeks	(a)	—	—
	(b)	97.06 ± 2.45	237.48 ± 14.79
	(c)	96.03 ± 2.25	151.56 ± 9.87

(a) 267 Rad/fraction (b) 205 Rad/fraction (c) 167 Rad/fraction

^{3}H thymidine was administered to pigs intradermally (10 μCi/0.1 ml) one hour prior to biopsy. E.O.T. end of irradiation period.

Thus in summary late radiation damage to pig skin, represented by dermal atrophy would appear to be caused by changes in the vasculature which can be measured 3-4 months after irradiation. The disassociation of vascular changes in the dermis from early effects on the epidermis can be clearly illustrated by the fractionation of the radiation dose. Protracted daily doses enhance epithelial effects.

The results achieved are in keeping with the objectives set out in the original proposal. There is now a clearer understanding of the mechanisms involved in the development of early and late radiation damage to the skin. Such an understanding is essential if precise protection criteria are to be established.

Title of the project nr. 2. The pathogenesis of late vascular damage in the hamster cheek pouch.

Head of Project and scientific staff : J.W. Hopewell, Y. Gunn and D. Campling

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Radiation induced changes in the vascular system are believed to play an important role in the pathogenesis of late radiation damage to normal tissues. This has been acknowledged in many investigations into the pathogenesis of late radiation damage, and is reflected in our own studies in pig skin (Project No. 1).

The separation of primary vascular damage from secondary damage to the parenchyma is often difficult in complex organs. Hence, there is a need to examine such changes and the underlying mechanisms in a simpler vascular system. Such a simple model is to be found in the vasculature of the hamster cheek pouch. Each animal also has two pouches making it possible to make comparative measurements (irradiated/control) in the same animal, thereby minimising one potential source of variability.

The effects of single doses of 250 Kv x-rays (HVL, 1.4 mm Cu) in the range 500 - 2500 rad are being studied for periods of up to 24 months following irradiation. Sixteen week old male Syrian hamsters were used and the left pouch was irradiated. The other pouch and the rest of the animal was shielded by 4 mm of lead.

At regular intervals after irradiation the animals were anaesthetised and each pouch everted, in turn, over a microscope stage, several photographs of different areas of each pouch were taken.

The main method used to quantify the observed vascular changes has been to measure the vessel wall/vessel volume ratio using a standard test grid placed over photographs. The hit/cut ratio as it is frequently called is a function of vessel diameter, hence any alteration in the distribution of blood vessels of different sizes will be reflected by a change in this ratio.

Estimates of the surface/volume ratio were made at monthly intervals for the first 9 months and less frequently thereafter. Relative values for irradiated/control pouches are plotted in Fig. 1. The data is incomplete as it is proposed to follow the animals for periods up to 24 months after irradiation.

The available data would suggest that even after a dose of 500 rad there was a significant reduction in hit/cut ratio in the irradiated pouch 4 months after irradiation. This was a transient change and by 5 months there was complete recovery with no further observable changes up to 12 months.

Surprisingly after 1000 rad no significant alterations in the hit/cut ratio were observed in the first 9 months after irradiation, a result comparable with that seen after a higher dose of 1500 rad.

After higher doses, 2000 - 2500 rad, two waves of modification in the hit/cut ratio were found. Both changes would seem to be transient with peak depressions at 3 and 9 to 12 months respectively.

The first transitory depression in hit/cut ratio seen at all dose levels (except 1000 and 1500 rad) correlates well with the reduction in blood flow noted in earlier functional studies (Hopewell, 1975, Radiat. Res. 63, 157). The absence of response after 1000 rad is as yet unexplained, but it is in agreement with the results of the earlier functional studies.

The timing of this first depression in the hit/cut ratio corresponds with the second wave reaction described in the pig skin studies. This seems likely to represent a truly primary vascular response. The later changes seen with the higher doses from which there may be an incomplete recovery, are not yet fully understood.

The first wave reaction would appear to be of importance in the aetiology of late damage. When the first wave depression was at its peak, microscopic observations frequently showed the existence of 'sausage' segments in small arterioles after doses $\geq$ 2000 rad.

Therefore, autoradiographic studies have been initiated to obtain a fuller understanding of this phenomenon.

The reduction of the hit/cut ratio is thought to be due to a specific loss of the capillary microvasculature, and not a general depletion of blood vessels. The distribution of vessel diameters in the normal pouch shows a peak at 7μm. Work is underway to determine the changes in size distribution of vessels in the irradiated vasculature at specific time periods. These should be of further value in evaluating the response of the vasculature to radiation.

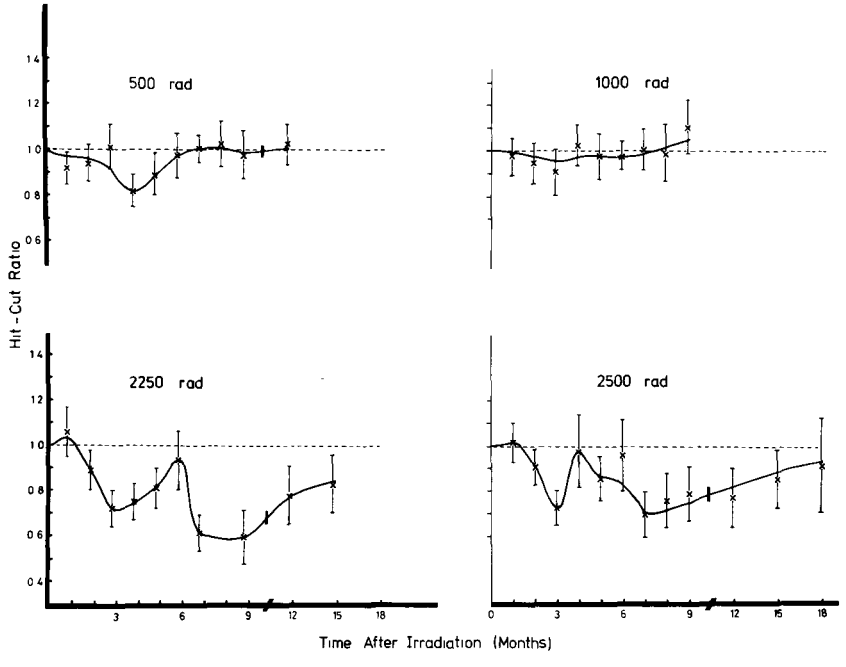

Fig. 1. Relative changes (irradiated/control) in the vessel hit-cut ratio in the hamster cheek pouch as a function of time after irradiation with single doses of 500 to 2500 rad. Error bars indicated one standard error. Error on control values ± 4%.

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Hopewell, J.W. and Young, C.M.A. (1978). Changes in the microcirculation of normal tissues after irradiation. Int. J. Rad. Onc. Biol. Phys. 4, 53-58.

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Contractor: College of Technology, Kevin Street, Dublin 8.

Contract No: 264/78/1 BIO EIR

Head of Research Teams: J. F. Malone and M. J. Cullen.

General Subject: Dosimetric and Radiobiological Investigations of Iodine Radionuclides in the Human Thyroid.

Work on this project commenced half way through the last programme and was funded at a very modest level. However, the project has been very successful when considered in relation to its initial objectives and in relation to thyroid radiation protection in general. First a new method of monitoring iodine kinetics in the gland was evolved, validated and demonstrated to be applicable in practice. The method, based on applying thermoluminescent discs to the neck surface, greatly simplifies the practical aspects of acquiring kinetic data by removing the need for the subject to visit a laboratory to be counted. Further to this the many formulations and sets of physical data used in thyroid dosimetry were reviewed to select the most appropriate system for use today. The results of this review are presented in the monograph referred to in Publication 10.

As well as dosimetry this project was concerned with the radiobiological response of the thyroid. Following observation of clear dose-effect relationships for hypothyroidism in humans it was decided that it would be worthwhile developing a model of the thyroid in which radiobiological effects could be studied. This took the form of a cell culture system in which many of the morphological and functional properties of the gland were preserved. The model was exploited to demonstrate the effect of single doses of X-rays on cell survival, follicular morphology, and cell function, features which were for the first time clearly observed with this system. Having and developing such a model system is obviously very important with regard to understanding the radiobiological response of the gland. Apart from the observations listed above the implications of ICRP-26 for the thyroid were explored in detail and indicate the desirability of additional work in this area.

Results of Project: No. 1

Head of Project and Scientific Staff: J. F. Malone, M. J. Cullen, C. Mothersill, M. K. O'Connor, A. Murphy.

Title: Dosimetric and Radiobiological Investigations of Iodine Radionuclides in the Human Thyroid.

In the course of work on these projects results in several areas were obtained whose significance is summarized on the previous page. The main areas of investigation were.

1. The thyroid uptake measurement.

Studies of the influence of thyroid geometry on uptake measurements with ^{131}I, ^{125}I and $^{99}Tc^{m}$ were performed. The results revealed that serious errors can arise because of variations in gland depth and that gland mass or lobe separation do not cause large errors. Methods of correcting for gland depth based on counts taken at two different working distances were developed and validated. Measurements of gland depth in a group of patients were made and demonstrated a substantial variation from person to person.

2. Measurement of Iodine Kinetics with Thermoluminescent Discs.

Detailed studies of the response of the discs to ^{125}I and ^{131}I in a neck phantom were performed. These defined the limits of sensitivity and accuracy of discs that might be used to monitor iodine kinetics in the human thyroid. The results demonstrated a sensitivity that was compatible with monitoring doses administered to human subjects in the μCi range. They also indicated less dependence on the thyroid - neck geometry than might be expected. The effectiveness of the disc method for iodine kinetics in human subjects was demonstrated in a study in which comparison with results from an external probe was performed. Excellent agreement between the two methods was obtained. The range of effective half lives observed in thyrotoxic patients varied from about 2 - 7.5 days.

3. Thyroid Mass Estimation.

A survey of the literature in 1978 revealed that at the time mass estimates for large glands by palpation, scintiscanning and ultrasound gave results of similar accuracy when skilled experienced observers made the estimates. For smaller glands it is likely that ultrasound and CAT scanning will give more accurate results. With ultrasound use of a high frequency transducer with short focal length is important and the advent of digital scan converters will greatly reduce the labour involved in gland mass estimates.

4. Radiobiological Observations.

(a) Tissue Culture System: A tissue culture system for sheep thyroid cells which retains differentiation for up to 50 days has been established. This allowed radiobiological experiments be performed with isolated thyroid cells. The results indicated that non proliferating cells can survive for a long time even after 9,000 rads gamma rays without depopulating or de-differentiating. Proliferating cells exhibit a dose response relationship for cell survival typical of mammalian cells with a D_0 value of about 400 rads and a low extrapolation number. Iodine trapping is sensitive to radiation damage. The development of this damage is time dependent and reaches a plateau value at a dose of about 2,000 rads of gamma rays.

Some of the aspects of the culture system that allow differentiation be maintained are dependent on the energy metabolisms cycle of the cells with prominent roles associated with glucose and lactate metabolism. These features as well as contributing to differentiation may also be important in regulating aspects of the radiation responses.

(b) Human Systems.

An analysis of the follow up data from patients treated for thyrotoxicosis revealed that the incidence of hypothyroidism two years after therapy had a linear dose - effect relationship. This relationship was confirmed for ^{125}I and a wide range of different studies with ^{131}I reported in the literature. A hypothesis in incidence of hypothyroidism after radioiodine therapy has been developed.

The consequences of ICRP-26 for thyroid radiation were examined in detail particularly with regard to dose limits and consequent non fatal harm. It was felt that this area largely ignored by ICRP needs more serious attention.

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Contractor :	National Radiological Protection Board, Harwell, Didcot, Oxon. OX11 ORQ England
Contract No. :	179-76-1 BIO UK
Head of the research team :	Dr. J. A. Dennis and Dr. H. Smith
General subject of contract :	The movement of actinides in mammals

Title of Project No. 1 :	The binding of actinides to mammalian proteins and other biologically occurring molecules
Head of Project and scientific staff	Dr. D. S. Popplewell, Dr. G. N. Stradling, Dr. J. R. Cooper

The major route of intake of actinides into the body is through the lungs. Some of the inhaled material eventually reaches the blood and it is then deposited either in body tissues, particularly bone and liver, or it is excreted. One of the problems in radiological protection is to explain the differences that exist in the rates of clearance of actinide compounds from the lungs and to explain why urinary excretion may or may not be the primary route of excretion.

Our experiments have shown that the amount of actinide oxide moving from the lungs to blood is related to the proportion of small (0.001 μm diameter) particles present in the inhaled dust, or in the rate of formation of these small particles while the larger particles remain in the lungs. The actinides studied include $^{239}PuO_2$[1], $^{239}PuO_2$ mixed with Na_2O[2], $^{238}PuO_2$[3], $^{241}AmO_2$[4] and $^{244}CmO_2$[5].

The movement of positively charged small $^{238}PuO_2$ particles from lungs to blood is influenced by their affinity for phospholipids which are present in lung surfactant[6]. The ^{238}Pu-phospholipid complex is taken up by alveolar macrophages and is in fact avidly retained in the macrophages. Small unattached particles would readily cross the lungs to blood[7,9] so that there is a delay in the rate of transfer when the phospholipid complex is formed.

In contrast, negatively charged small $^{244}CmO_2$ particles do not react with the constituents of lung surfactant and they diffuse passively into the blood[10]. On entering the blood, they react predominantly with serum proteins and are

then transported to sites in bone and liver. This process can be inhibited by injecting DTPA into the blood with the result that the small particles are excreted unchanged in the urine instead of depositing in the tissues[11]. This procedure is much more effective than artificially increasing serum levels of citrate and succinate ions[12].

Any actinide reaching the blood in soluble form, as distinct from small particulate, rapidly reacts with serum protein and to a lesser extent with serum citrate ions[13,14]. The low molecular weight citrate complex is filtered through the kidneys while the actinide associated with the protein is transferred into cells to be bound to intracellular proteins. Some biliary excretion of soluble carbonate complexes can also occur[15] but in the case of plutonium the mechanism is of minor importance.

These studies have provided a rational basis to explain the variations that exist in the urinary excretion of actinides following their intake into the body and to suggest better ways of enhancing their excretion from the body.

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Title of Project No. 2 : Development of new chelating agents for removing plutonium from intracellular sites

Head of Project and scientific staff : Dr. D. S. Popplewell, Dr. R. A. Bulman, Dr. J. D. Harrison

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There is a pressing need in radiological protection to provide a safe and effective technique for enhancing the elimination of actinides deposited in body tissues. At present, the only recognised procedure is to administer the chelating agent diethylene-triamine-penta-acetic acid (DTPA), either intravenously or as an aerosol. It is unfortunate that DTPA so administered is only partially effective for soluble plutonium and almost totally ineffective for insoluble plutonium. One reason for this is that the chelating agent is hydrophilic and cannot pass through cell membranes to reach intracellular deposits of plutonium.

On the basis that lipophilic molecules can cross cell membranes, various derivatives of DTPA and ethylene diamine tetra-acetic acid (EDTA) were prepared. It was considered likely that the replacement of the hydroxyl part of some carboxyl groups in the molecule should result in a greater lipophilicity of the molecule without altering its ability to chelate plutonium with the remaining intact carboxyl groups.

Mono- and di-glucosyl-amido derivatives of EDTA, mono-leucyl-amido-EDTA, mono-oleyl-amido-EDTA, mono-dodecyl-amido-DTPA and ester derivatives of the alcohols corresponding to the amino-alkanes were synthesised. None of these compounds, when administered to hamsters, resulted in the increased excretion of plutonium from the liver.[1,2,3] However, a lipophilic eompound, code-named Puchel (Figure 1), was prepared, which proved to be partially effective.[4]

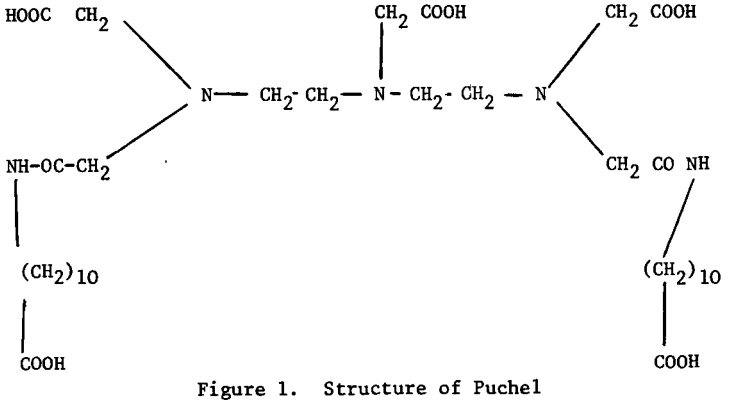

Figure 1. Structure of Puchel

Initial studies indicated the delayed effectiveness of Puchel when it was injected intra-peritoneally into hamster 3 days after an injection of ^{239}Pu citrate (Table 1). This effectiveness was much less in the rat.

Table 1 Percentage of injected plutonium recovered from hamsters

Treatment	In excreta	In liver
DTPA (50 mg kg^{-1})	2	13
Puchel (50 mg kg^{-1})	33	2
None	1	19

It was also shown to be partially effective in removing plutonium from lungs if ^{239}Pu nitrate was injected into the lungs to be followed at various time intervals by aerosol of Puchel (10 mg kg^{-1}) (Table 2).

Table 2
Percentage of intubated plutonium retained

Tissues	Controls	Puchel administered after a delay of			
	7d	1d	2d	5d	7d
Lungs	41.2	11.7	12.5	11.3	14.5
Liver	17.2	7.4	2.4	6.0	9.1
Carcass	32.0	20.0	17.7	27.3	32.3
Total	90.4	39.1	32.6	44.6	55.9

For comparison, 30% of the administered plutonium was retained in the lungs if DTPA was administered in aerosol form after a delay of 7 days. Furthermore, any plutonium excreted after administering Puchel was found in the faeces, whereas it was excreted in the urine after DTPA administration.

To study the tissue distribution of Puchel, a tritium-labelled form was injected into hamsters and rats. It was rapidly cleared from hamster liver but not so readily from rat liver. This may explain the enhanced clearance of plutonium from hamsters relative to the rat. Preliminary studies on the toxicity of Puchel are progressing. At the doses expected to be of therapeutic value, there is no evidence of serious tissue damage.

Puchel has been shown to be partially effective in removing plutonium and americium oxides from the lungs of hamsters. It was not effective in removing plutonium or americium from bone. Some effort was directed to preparing chelating agents which were expected to have a strong affinity for bone. Two compounds dodeca-phosphonate of docosone and a diphosphonic acid derivative of DTPA were synthesised and administered to rodents previously injected with a soluble form of plutonium. Neither compound enhanced the removal of plutonium from bone.

At present the synthesis of new chelating agents is no longer being pursued. Current work is devoted to optimising treatment regimes involving Puchel.

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Title of Project No. 3 : Experimental studies on the movement of actinides from wound-simulated sites

Head of Project and scientific staff : Dr. J. W. Stather, Dr. J. D. Harrison

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Wound contamination with materials containing actinides is a hazard in the reprocessing of irradiated fuel elements and in the purification of actinide elements. Studies of the physical and chemical factors affecting retention in wounds and movement to other tissues have been carried out in laboratory animals.

Wound contamination with various chemical forms (e.g. citrates, nitrates, oxides) of protactinium, plutonium, americium and curium was simulated by either intramuscular or subcutaneous injection in rodents and rabbits[1,3,4].

In general terms, it was shown that soluble forms of the actinide rapidly reached the blood to be distributed primarily in bone and liver in a similar way to that observed after the intravenous injection of the actinide. Insoluble material moved much more slowly through the lymphatic system, draining from the tissues surrounding the wound. Most of the activity was trapped in those lymph nodes nearest to the wound but a small amount reached the liver in particulate form[1,2,3]. Any material retained in the wound site was gradually encapsulated in fibrous tissue but no neoplastic changes were observed in any of the animals examined[4].

Various forms of treatment with chelating agents have been used to influence the movement of plutonium from wounds[5]. Localised injection of diethylene-triamine penta-acetic acid (DTPA) into the muscle tissue surrounding an intramuscular deposit of hydrolysed plutonium was shown to be more effective than an intravenous injection in clearing plutonium from the contaminated tissue. DTPA combined with another chelating agent, sodium citrate, was more effective than DTPA alone; and the inclusion of the vaso-dilating agent, nicotino-hydroxamic acid, further enhanced the clearance.

Blood flow is an important factor in influencing clearance. When compounds known to reduce capillary blood flow were injected, namely the anti-inflammatory agent, Bufexamac, and the wound healing agent, calcium acexamate, plutonium clearance was markedly reduced.

The effectiveness of the partially lipophilic derivative of DTPA (Puchel) was compared to DTPA[6]. When injected locally into muscle tissue surrounding the plutonium, both were equally effective when injected 15 minutes after the injection of ^{239}Pu nitrate; but Puchel was considerably more effective when the injections were delayed for 5 days. However, the increased clearance of plutonium after Puchel injection was accompanied by a greater deposition of the circulating plutonium in liver and bone. This may be a contra-indication for the use of Puchel under these circumstances.

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Title of Project No. 4 : Lung clearance and translocation of inhaled industrial dusts

Head of Project and scientific staff : Dr. J. W. Stather, Dr. A. C. James, J. C. Strong, Dr. N. D. Priest .

The lung clearance and tissue distribution of some actinides has been followed after exposing animals to materials to which workers in the nuclear industries may be exposed, or which could be released into the environment in the event of an accident. To undertake these studies, a small animal inhalation facility was constructed. This allowed 'on line' monitoring of the dust concentration in the exposure chamber and precise control of the exposure conditions[1]. The materials investigated included $^{238}PuO_2$, $^{241}AmO_2$, and dusts containing mixtures of plutonium, americium and uranium oxides obtained from a glove box in a production line for experimental fast reactor fuel.

Groups of hamsters exposed to $^{238}PuO_2$ were studied for up to 600 days following exposure at two levels (1.1 kBq and 7.3 kBq initial lung deposit, I.L.D.)[2,3]. Approximately half of the I.L.D. was cleared with a half-time of about 20 days. Long-term retention of the remainder depended upon the I.L.D. At the low level, the half-time of long-term retention was about 240 days; at the highest level, it corresponded to over 1000 days. The difference in retention characteristics was related to the degree of fibrosis in lung tissue, which increased in severity according to the radiation dose.

A feature of these experiments was that more ^{238}Pu moved from lungs to blood than the corresponding amount of ^{239}Pu following exposure to $^{239}PuO_2$[2,4]. The extrapulmonary tissue deposit of ^{238}Pu increased with time up to about 3 months after exposure, when the amount translocated to the liver and carcass was about 7% of the I.L.D. Thereafter, there was little further translocation. This is surprising in view of the fact that the $^{238}PuO_2$ was shown to fragment continuously to small (0.001 μm diameter) particles when suspended in water at room temperature. The explanation is that this fragmentation process also occurred in the lungs, but the small particles were trapped in alveolar macrophages and could not therefore be transferred to blood. Thus $^{238}PuO_2$ could be classified as a Class Y compound, as defined in the ICRP lung model

The lung clearance of plutonium and americium in hamsters exposed to a dust consisting of particles of PuO_2 (+ AmO_2) and UO_2 (ratio 1:2 by mass) was measured[4]. At the time of exposure, about 10% of the activity was due to ^{241}Am and the remainder to plutonium isotopes (^{238}Pu, ^{239}Pu, ^{240}Pu). All but a small fraction of the activity cleared from the lungs was excreted in the faeces[2].

By 540 days after exposure, when the lungs retained about 10% of the ILD (800 Bq), the amounts of plutonium in the thoracic lymph nodes and in the extrapulmonary tissues (mainly skeleton and liver) were respectively 0.5% and 0.2% of the ILD. This is much less than would be predicted for a Class Y compound and therefore the risks to bone and liver are also much less.

The lung clearance of ^{241}Am did not differ appreciably from that of $^{238,239,240}Pu$, indicating that it was not selectively leached out of the PuO_2 particles in the lungs. The levels of ^{241}Am in other tissues were comparable to those of $^{238,239,240}Pu$ (<1% of the ILD). This observation is in contrast to results obtained in hamsters that had inhaled $^{241}AmO_2$ alone, when at 275 days after exposure, up to 45% of the ILD of ^{241}Am had translocated to extrapulmonary tissues while 13.5% remained in the lungs[2,5]. Electron micrographs of samples of $^{241}AmO_2$ sampled at various times after suspension in water showed that the initial crystalline structure rapidly became amorphous and this no doubt accounted for the high solubility of the material in the lungs[5].

These studies have demonstrated how both particle size and chemical form can influence the behaviour of actinides in the lungs. They show that if an actinide is inhaled as a minor constituent in combination with other elements it is the element present in the greatest mass that largely determines the behaviour of the minor constituent; and that for some PuO_2 aerosols likely to be inhaled by workers, the ICRP lung model currently used to estimate tissue doses may overestimate the amount of plutonium translocated to the bone and liver.

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Contractor :	National Radiological Protection Board, Harwell, Didcot, Oxon. OX11 ORQ England
Contract No. : :	182-76-1 BIO UK
Heads of Research Team :	Dr . J. A. Dennis and Dr. H. Smith
General subject of contract :	The dosimetry of internal emitters

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Title of Project No. 1 :	The deposition and clearance of radioactive aerosols in animal lungs
Head of Project and scientific staff :	Dr. A. C. James, Mrs. J. R. Greenhalgh

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Reliable experimental data are required on the deposition andclearance patterns of sub-micron sized particles in lungs. Using these data, it is possible to construct metabolic models to predict the dose to radiation-sensitive cells in the airways of humans exposed, for example, to short-lived α emitters such as radon and thoron daughters[1,2,3].

In an initial experiment, the deposition of radon daughters attached to condensation nuclei was measured in artificially ventilated, excised pig lungs under conditions of airflow and humidity approximating those existing in human lungs[4]. Radioactivity was deposited in the upper airways in accordance with theoretical predictions based upon a knowledge of Brownian diffusion of particles under steady laminar flow conditions in tubes. However, when free lead ions (^{212}Pb) were introduced into the lungs, deposition of radioactivity in the upper airways was less than that predicted by a factor of about four. This was consistent with the view that small ions were absorbing water from the humid air in the lungs, growing in size and therefore depositing less efficiently in the upper airways.

Clearance of radioactive particles from the airways involves two mechanisms. They can move up to the trachea and mouth in mucus, a process which is aided by the ciliary action of cells lining the airway; or the radioactivity associated with the particles can transfer from the surface of the airways to the blood. The latter process is not well understood, yet there is no doubt that it is a significant means by which radiation-sensitive cells in the airways are irradiated.

In experiments with anaesthetised rabbits, some factors influencing the transfer of lead ions from the airway epithelium to blood were investigated[5,6,7]. About 10% of the radioactivity associated with the deposited particles was transferred to blood with a half-time of 17 ± 5 min; the remainder transferred with a half-time of about 9 hours, irrespective of where it was deposited in the airways. However, a marked difference was noted in the anaesthetised rat[8]. About 40% of the activity transferred to blood with a half-time of about 30 minutes. This could be increased to about 60% if inert lead carrier was added to the lead ions or under acid conditions; but it was drastically reduced (∿2%) if inert material (i.e. ferric hydroxide) capable of binding lead ions was present.

Muco-ciliary clearance remains the major route of removal of insoluble particles from the respiratory tract. This was demonstrated by the double labelling of mucus with micron sized insoluble γ tagged particles[9] and lead ions and measuring their relative movements in the nasal airways of rats[10].

A compartment model of epithelial retention and transfer processes was developed[11] using these data. About 75% of insoluble γ-tagged particles introduced onto ciliated nasal epithelium were cleared to the mouth and then the gut with a half-time of 12 minutes (range 7-18 min); the remainder stayed in the airways, probably in slowly moving streams of mucus.

A smaller fraction of lead ions (∿60%) was cleared to the gut with a half-time similar to that observed for insoluble particles. However, rapid uptake of ^{212}Pb to blood was observed. About 8% of deposited ^{212}Pb transferred to blood with a half-time of approximately 12 minutes and about 30% remained in the nasal airways. Since the dose to the radiation-sensitive cells particularly in the upper airways is critically dependent on whether radon daughters remain in mucus or are retained in the epithelium, these data emphasise the need to incorporate both prolonged and short-term retention in the epithelium in any dosimetric model.

This experimental work, together with analysis of published data on aerosol deposition in human lungs, has provided a data base from which a radon daughter deposition, clearance and dosimetry model for the human airways has been developed. The results of modelling have contributed to the work of the NEA Expert Group on Radon Dosimetry and Monitoring[2,3] in their preparation of a "State-of-the-Art" report on this subject.

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Title of Project No. 2 : Pulmonary deposition and clearance characteristics of inhaled particles in human volunteers

Head of Project and scientific staff : M. R. Bailey, Dr. A. C. James, F. A. Fry

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Quantitative data on the retention patterns of particles in human lungs are required to assess radiation doses incurred to sensitive cells when radioactive materials are inhaled. Since experiments with potentially hazardous materials cannot be carried out on humans, the retention of a non-toxic, relatively insoluble material (fused aluminosilicate particles, FAP) was studied in man, and has been compared with that of highly toxic $^{239}PuO_2$ in rodents.

A technique was developed for producing γ-tagged FAP of uniform size[1,2,3]. Montmorillonite clay in aqueous suspension is labelled by ion exchange. A spinning-top generates uniform droplets of the suspension which are dried, concentrated into a low airflow by inertial separation, and heated to 1200°C, producing aluminosilicate glass microspheres. Monodisperse (geometric standard deviation <1.2) particles of diameter between 1 μm and 5 μm can be prepared with a yield >10%. Fractional dissolution rates measured *in vitro* were in the range 10^{-4} to 10^{-3} d^{-1}[4,5].

To assess the *in vivo* dissolution rate, FAPs labelled with ^{88}Y ($t_{\frac{1}{2}}$ 107 d) or ^{85}Sr ($t_{\frac{1}{2}}$ 65 d) were administered to rats by inhalation; and compartment models for the behaviour of ^{88}Y and ^{85}Sr in the rat were constructed using the results of experiments in which the labels were injected intravenously as chlorides[9,10]. About 0.1% of the initial deposit of FAP dissolved in the first day and entered the blood. Thereafter the dissolution rate varied between 10^{-4} d^{-1} and 10^{-3} d^{-1}, in good agreement with the *in vitro* values.

The retention pattern in the lungs was also followed in these animals for up to 460 days[6,7,8]. The results can be best fitted by a two-compartment exponential curve with clearance half-times of 20 d (60%) and 170 d (40%). However, a species variation was found to occur. ^{85}Sr-FAP clearance from the lungs of hamsters (up to 350 d after inhalation), followed a single exponential with a half-time of 100 d.

The clearance of $^{239}PuO_2$ [11], initially similar to that of FAP (up to 250 d in rats and 50 d in hamsters), became significantly slower, probably as a result of damage to the lungs.

Apparatus for administering particles to human volunteers by inhalation under controlled condihions was designed and constructed. The subject inhales a known concentration at a fixed inhalation rate and tidal volume. Procedures were tested using FAP labelled with ^{67}Ga ($t_{\frac{1}{2}}$ 3 d) and it was shown that the pulmonary deposit could be controlled to within $\pm$ 50%.

Seven healthy, non-smoking male subjects inhaled 1.2 μm diameter ^{85}Sr-FAP and 3.9 μm ^{88}Y-FAP [12]. Particle retention was determined at appropriate intervals by external counting of γ-rays using an array of NaI (Tl) detectors in the NRPB low-background facility. Approximately 8% of the 1.2 μm and 40% of the 3.9 μm particles cleared within 6 days. It was calculated that these fractions were deposited in the conducting airways and no evidence was found for the rapid phase of pulmonary clearance (40% removed with a 1 day half-time) assumed in the latest ICRP lung model. Approximately 3% of the 1.2 μm and 15% of the 3.9 μm particulate retained at 6 days cleared with a half-time of about 20 d, the rest with half-times averaging 320 d and 520 d respectively, in agreement with the 500 d half-time for the slow phase of pulmonary clearance of insoluble particles chosen for the ICRP lung model. There was, however, considerable inter-subject variation, the highest clearance rate being twice the lowest. Lung dissolution half-times were estimated to be 700 d and 1400 d for the 1.2 μm and 3.9 μm particles respectively, indicating a half-time of about 700 d for the slow phase of pulmonary clearance of particles, presumed to be contained in macrophages.

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Title of Project No. 3 : Internal dose from carbon-14 labelled compounds

Head of Project and scientific staff : Dr. J. W. Stather, Dr. F. E. H. Crawley

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Carbon-14 labelled compounds are used frequently in industry and medicine. The objective of this study was to provide a basis for assessing the radiation dose in human tissues from some of these compounds.

The compounds chosen initially were selected from a group of elementary precursors used in preparative procedures for drugs and other biochemicals. These are the building blocks for compounds of more complicated structure. They are of high specific activity and even a small release of radioactivity can involve the intake of a relatively large amount of carbon-14.

Metabolic studies in rodents were completed with carbon-14 labelled potassium cyanide, methyl alcohol, sodium acetate, barium carbonate, nitrobenzene and glycine.[1,2,3] These compounds were administered intravenously, intra-gastrically, by pulmonary intubation or directly on to intact skin. The distribution and radiation doses of up to fifteen different organs and tissues were determined. These data were then extrapolated to predict the radiation doses to man. For all compounds studied, the effective dose equivalent was calculated to be in the range $2.5 - 4 \times 10^{-10}$ Sv Bq^{-1} and the Annual Limits of Intake were estimated to be within the range $1.2 - 2 \times 10^{8}$ Bq. Extensive data on the excretion of these compounds after different routes of entry has provided information that can be used for interpreting the results of bioassay data obtained from persons occupationally exposed to these compounds.

The experimental work was later extended to include a study of the metabolism of ^{14}C labelled diethylene triamine penta acetic acid (DTPA), a chelating agent used to enhance the excretion of heavy metals, including plutonium, from the body. Preliminary experiments were undertaken in rats to provide the basis for a human study in which the labelled drug was administered to volunteers by intravenous injection or inhalation. The effective dose equivalent calculated from the rat data for various organs in man was 5.4×10^{-11} Sv Bq^{-1} excluding the lower large intestine (LLI) which received the highest dose. After intravenous injection the dose equivalent to the LLI was 3.8×10^{-10} Bq^{-1}, and after deposition in the pulmonary region of the lung 7.3×10^{-10} Sv Bq^{-1}. It was noteworthy that about 15% of the DTPA was excreted in the faeces after intravenous injection, presumably as a result of secretion into the bile.

In two human volunteers most of an intravenous injection of ^{14}C-DTPA (~98%) was cleared from the body with a half-time of about an hour. Urinary clearance could be described by a 3 component exponential function with half-times of 0.025 d (65%), 0.076 d (34%) and 1.7 d (0.25%). The activity detected at 7 days was 0.02% of the administered level. This reflects an amount circulating in blood which would still chelate plutonium and could explain the enhanced urinary excretion of plutonium that is found several days after treatment in persons given DTPA. In contrast to the study with rats, all (>99%) of the DTPA was excreted in the urine. The total activity injected into each volunteer was 1.3×10^{6} Bq giving an effective dose equivalent estimated to be 2.7×10^{-7} Sv. Each volunteer then inhaled about 200 mg Ca-DTPA (1.1×10^{6} Bq ^{14}C-DTPA) in 15 minutes. About half of this material subsequently translocated to the blood with a half-time of about 1 hour. As the half-time of retention of DTPA in the blood was also about 1 hour inhalation of the chelating agent effectively doubles the time over which a therapeutically useful amount of DTPA is retained in the body compared with intravenous injection. The dose equivalent to the lungs of each volunteer was estimated to be approximately 3.0×10^{-11} Sv Bq^{-1} deposited in the lungs.

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Contractor : CNEN
Contract Number : 174-77-1-BIOI
Program Leader : Dr. G.F. Clemente
General Subject : Experiments on long term effects (bone carcinogenesis) induced by plutonium in mice.

Final Report for Project n. 1
Project Leader: Dr. G.F. Clemente
Title of the Project: Dose-incidence relationship for bone tumours at low body burdens.

The need for further data on biological effects of plutonium contamination at low doses on various animal species is evident mainly because the existing data have led to some contradictory interpretations.

Current experimentation on dogs at low doses are under way and the final information will be available only within several years. Recent data published in the Annual Report (1) of the Radiobiology Division of Univ. Utah seem to show that a significant incidence of bone sarcomas induced by plutonium in dogs may exist at injected doses in the range of 0.0018-0.016 uCi/kg body weight.

Few data on rodents at low plutonium doses are available to date and mostly on rats; therefore new data on mice could be of high value to assess the differences, if any, among various animal species, in the shape of the dose-response curves for low doses.

Considering the bone sarcoma induction in various animal species by low doses of alpha emitting radionuclides, the shape of the dose response seems to be concave upwards for 228 Ra in beagles, concave downwards for 224 Ra in mice, linear for 226 Ra in mice and "approximately" linear for 228 Th in beagles, 239 Pu in rats, 239 Pu in mice, 227 Th in mice (1).

The summary of the experimental plan undertaken under this project is given in table 1.1. The mice have been injected at doses in the range of 0.0012-0.12 uCi/kg body weight which is very similar to the range of low doses injected to the Utah beagles (1).

The average skeletal content at the end of life expectancy (about 750 days) has been also evaluated on the basis of the skeletal retention function for plutonium observed in our mouse strain (see project n. 2).

The only available data on 239 Pu induced bone sarcomas in mice have been observed by Finkel and Biskis (2) in the range of 0.16 - 15.6 uCi/kg body weight injected doses; bone sarcomas have not been observed in the range of 0.04 - 0.08 uCi/kg body weight in mice showing an average survival time of about 450-500 days only. Such short survival time was mainly due to the high incidence of intestinal infection in all animal groups (2).

The number of expected bone tumours in our animal groups has been also given in table 1.1. on the basis of the Finkel and Biskis data.

Table 1.1

Summary of the experimental plan for the study of the induction of bone carcinogenesis by plutonium at low doses in mice.

Number of injected animals	Injected Amount per animal		Skeletal Content° at the end of life expectancy		Skeletal Average Dose °° at the end of life expectancy	Number of expected bone sarcomas °°°	
	(pCi)	(uCi/kg)	(pCi)	(pCi/g)	(rad)	(%)	Total per group
500	30	0.0012	3.6	2.0	0.6	0.07	0.1
500	150	0.006	18	10	3	0.3	2
400	300	0.012	36	20	6	0.7	3
400	750	0.030	90	51	15	1.7	7
400	1500	0.060	180	103	30	3.3	13
300	3000	0.120	360	206	60	6.7	20
Controls							
700	-	-	-	-	-	0.1 ^	0.1

° Based on the retention data reported in fig.2.1 for our mouse strain. Average skeletal weight equal to 1.75 g.

°° Based on average dose factor for our mouse strain of 500 rad to the skeleton at about 750 days after injection per uCi/kg body weight of injected dose.

°°° Based on the extrapolation at low doses of the bone sarcoma incidence (0.11 %per rad) observed by Finkel and Biskis (2) in mice injected with 239 Pu in the dose range of 0.16-15.6 uCi/kg body weight.

^ Based on the incidence of observed spontaneous bone sarcoma in our mouse strain.

During the period 1976-1978 the animal house and all other experimental facilities have been completed together with the development of the most important techniques needed for the implementation of the experimental plan shown in table 1.1.

The injection of the animals started during the second semester of 1978, beginning from the lowest level group.

About two thousand animals have been injected up to the end of 1980. All male animals are (C57 BL x C3H)F1 and have been injected intravenously by means of monomeric solutions of 239 Pu (1% trisodium citrate at ph 6.5).

At the end of 1980 the first animals from the lowest level group have begin to die. No pathologically demonstrated bone sarcomas have been detected until now in both the injected groups and the control animals.

All the animals are regularly followed-up until death and subjected to histophatological and radiographic examinations after death.
The following organs are collected during the autopsy for histophatological observations: femur, sternum, liver, kidneys, lungs, heart, spleen, g.i. tract and lymphnodes.

A fraction of the collected organs are stored for plutonium counting in case of positive histopathological observations. The dosimetry will be therefore performed for each individual animal showing a bone tumour.

Furthermore 10% of the total number of animals injected in each group will be selected at random and subjected after death to the determination of plutonium concentration in the following organs: right femur, liver, right kidney and right testis for the assessment of the average plutonium dose to the various organs of the animals of each group.

The x-ray examination of a mouse of the lowest injected group showing an alteration of the skull is given as an example in fig. 1.1.

Furthermore a microdosimetric model (see project 2) is also under development for our mouse strain to assess the variability of plutonium bone distribution as a function of time. The burial of plutonium in bone and its distribution among the various kinds of bone are in fact heavily influencing the total doses received by the osteoprogenitor cells lying on the bone surfaces of the injected animals.

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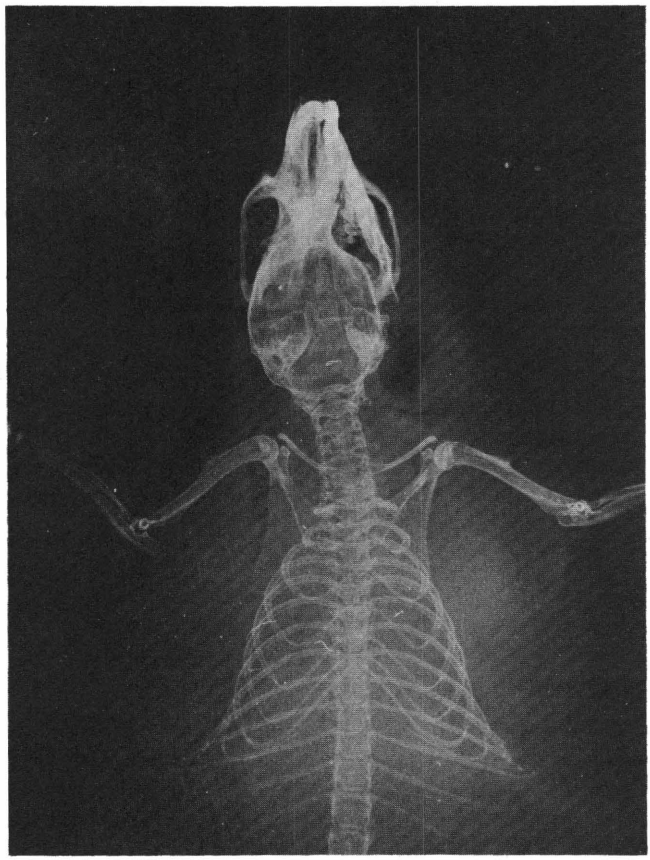


fig. 1.1. Radiographic examination of a mouse injected with 30 pCi of ^{239}Pu, showing an alteration of the skull.

Final Report for Project n. 2
Project Leader: Prof. P. Metalli
Title of the Project: The influence of physical and biological variables on the dose incidence relationship for bone tumours due to plutonium contamination in mice.

The main task of project 2 was related to the study of physical and biological parameters which can influence the plutonium dose absorbed by the various organs of mice. To this aim the plutonium distribution in femur, liver, kidney, and testes, and the retention function in the skeleton and liver have been studied in about fifty mice of our strain at various times post-injection and at injected doses of the order of 0.2-0.4 μCi/kg body weight. Higly sophisticated techniques have been developed to measure very low levels of plutonium in both the entire animals with and without liver (by the direct counting techniques) and in various tissues and organs (by radiochemical methods).

The results obtained have demonstrated that the direct counting of plutonium in mice by means of phoswich crystal is very powerful to study the retention function for long periods after injection at the low doses used.

A summary of the data on the distribution of 238 Pu and 239 Pu in various organs of our mice at various times post-injection(intravenous) of monomeric solutions similar to those used in the study of bone carcinogenesis (see project 1) is given in table 2.1. The data confirm that i) at about 100 days post-injection most of the remaining activity is in the skeleton, ii) the liver retention may be considered the main factor which affects the total body retention of plutonium in mice during the early period post-injection, iii) the plutonium fraction in the femur and kidney may be considered quite constant also long times after injection, iv) a constant fraction of about 0.06%is firmly retained in the male gonads of our mice for the entire life-span and v) significant differences between 238 Pu and 239 Pu may not be observed at injected doses in the range 0.2 - 0.4 uCi/kg body weight.

The distribution data given in table 2.1. are in good agreement with the existing data reported in table 6.1. of ICRP publication 19 (1) for mice injected with similar 239-Pu solutions and referred to a maximum period of 350 days post-injection.

The skeleton and liver retention functions have been plotted in fig. 2.1. together with the experimental points and related errors (at 95% level). All the data given in fig. 2.1. are pooled observations with 238 Pu and 239 Pu monomeric solutions intravenously injected in our mouse strain. The curves plotted in fig. 2.1. have been obtained by means of a best fit of the experimental points with the following equation:

$$R(t) = \sum_{i=1}^{3} A_i \, e^{-\lambda_i t} \quad (1)$$

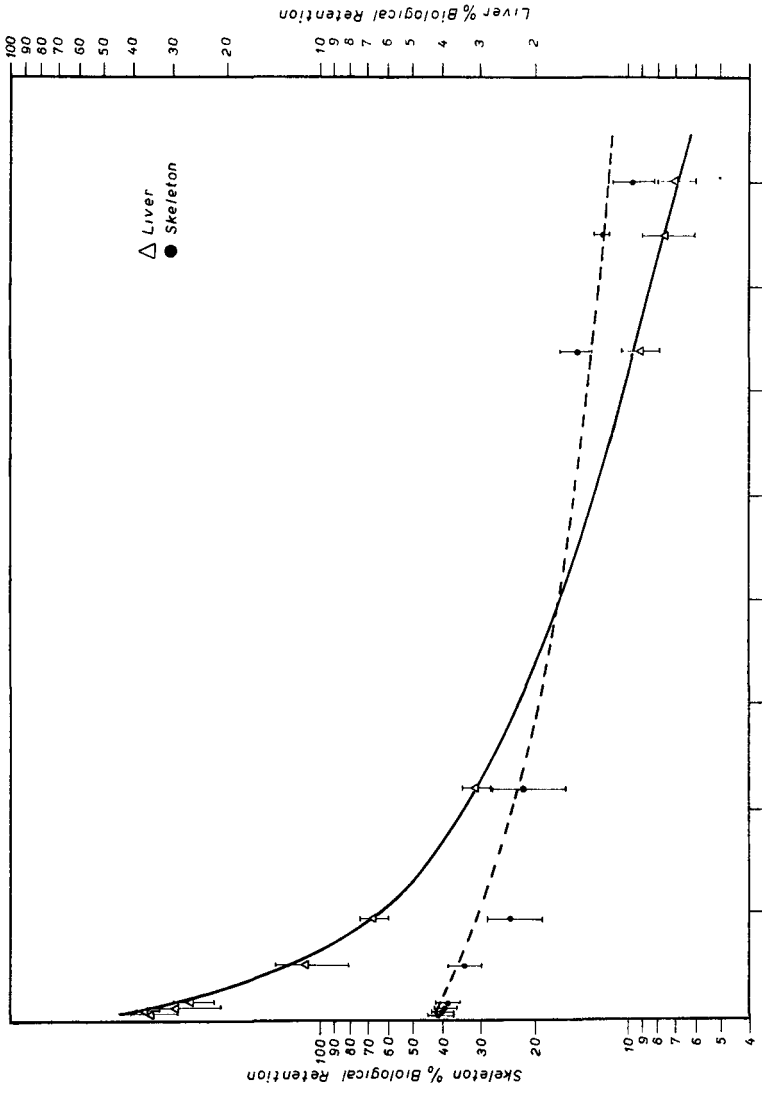
where for the liver retention function

$A_1 = 34.4$	$\lambda_1 = -3.98 \cdot 10^{-2}$	$T_1^{1/2} = 17.4$ days
$A_2 = 7.3$	$\lambda_2 = -9.25 \cdot 10^{-3}$	$T_2^{1/2} = 74.9$ "
$A_3 = 3.2$	$\lambda_3 = -1.96 \cdot 10^{-3}$	$T_3^{1/2} = 354$ "

Table 2.1.

Distribution of injected activity (percent of injected Pu ± 2 S) in various organs of (C57 Bl x C3H) F1 mice at various times post-injection.

Days post-injection	Right Femur		Liver		Right Kidney		Right Testis	
	^{238}Pu	^{239}Pu	^{238}Pu	^{239}Pu	^{238}Pu	^{239}Pu	^{238}Pu	^{239}Pu
10	1.5±0.4	1.1±0.2	28.5±5.5	32.8±6.5	0.11±0.02	0.12±0.02	0.03±0.01	0.04±0.01
50	1.4±0.1	1.4±0.2	12.9±1.1	8.5±1.5	0.10±0.01	0.08±0.01	0.03±0.01	0.03±0.01
95	1.0±0.3	1.0±0.5	7.0±0.6	6.4±0.4	0.07±0.01	0.05±0.01	0.04±0.01	0.03±0.01
220	1.1±0.1	0.7±0.1	3.0±0.3	3.2±0.2	0.06±0.01	0.07±0.01	0.03±0.01	0.04±0.01
640	0.7±0.1	0.6±0.1	—	0.9±0.3	0.06±0.01	0.06±0.01	0.02±0.01	0.04±0.01
750	0.5±0.1	0.5±0.1	0.6±0.1	0.9±0.1	0.05±0.01	0.04±0.01	0.03±0.01	0.02±0.01
800	0.46±0.05	—	0.7±0.1	—	0.04±0.01	—	0.02±0.01	—



The Biological Retention of Plutonium in Skeleton and Liver of (C57/BL x C3H)F1 Mice

fig 21

and for the skeleton retention function

A_1 = 22.9	λ_1 = $-5.9 \cdot 10^{-3}$	$T^{1/2}_1$ = 117.5	days
A_2 = 1.8	λ_2 = $-7.2 \cdot 10^{-4}$	$T^{1/2}_2$ = 959.2	"
A_3 = 16.8	λ_3 = $-5.9 \cdot 10^{-4}$	$T^{1/2}_3$ = 116 9	"

The retention data reported above are in good agreement with similar data summarized in table 7.1. of ICRP publication 19 where biological $T^{1/2}$ of the order of about 420 days and about 22 days are reported for skeleton and liver respectively, in mice injected with 239 Pu monomeric solutions.

Our data seem to show that a significant component with a quite long biological $T^{1/2}$ is influencing the plutonium liver retention in mice. Furthermore it is stressed the importance of the long term components of the plutonium skeleton retention which have a biological $T^{1/2}$ much longer than the average life span of the animals. These results may be considered of relevant significance from the radiation protection point of view owing to the contribution of the long term components of the retention functions to the total internal dose due to plutonium contamination. On the basis of the skeleton retention function given above and of the observed average life expectancy (about 750 days) of our mouse strain, the value of about 500 rads has been evaluated as the average dose received during the entire life span by the skeleton of the mice injected by 1 uCi/kg body weight of 239 Pu. Furthermore, for a better assessment of the dose received by the osteoprogenitor cells lying on the bone surface during the entire life span of the injected mice, some studies on the microdistribution of plutonium in the mouse skeleton are under way by means of a method based on neutron induced autoradiography. A collaboration on these activities has been conducted in the past and it is also planned for the future with the Radiobiology Units (Dr. G. Howells) of MRC at Harwell and the Institute for Genetic and Toxicology (dr. E. Polig) at Karlsruhe Nuclear Center.

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3. U. Andreozzi, G.F. Clemente, G. Ingrao, L. Mancini, G. Santori and Giovanetti A.E., Monomeric 239 Pu and 238 Pu rentention and distribution patterns in whole body and various tissues of BCF1 mice. Paper presented to the 14 th Annual Meeting of European Society for Radiation Biology, Julich -October 1978.

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9. F. Breuer, G.F. Clemente, E. Strambi, C. Testa: A case of internal contamination with plutonium oxide, in proc. of the 5 th Int. Conf. of IRPA, vol. II, pp. 99-102, Paris (1980).

Contractor : United Kingdom Atomic Energy Authority
Atomic Energy Research Establishment Harwell

Contract No : 104-76-1 PSTUK
Head of the research team : A. Morgan

General subject of contract : Deposition and clearance of inhaled particles in the human respiratory tract.

Final report of Project No 1 : Regional deposition and short-term clearance of inhaled particles in the human respiratory tract.

Head of Project and scientific staff : J. N. Pritchard, A. Black & M. Walsh

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The object of this programme was to obtain relevant data on the deposition and short-term clearance of aerosols inhaled by man, in order to facilitate improved estimates of radiological dose to the respiratory tract, which are currently based on the recommendations of the International Commission on Radiological Protection (ICRP). Using groups of healthy, male smoking and non-smoking volunteers it was planned to establish the normal range of intersubject variability and obtain information on possible effects on the respiratory system of cigarette smoking. This might, in turn, assist the early diagnosis of respiratory deficiency.

Method

Complete descriptions of the apparatus (Walsh et al., 1977) and of the procedures used (Foord et al., 1978) have been published. In brief, monodisperse polystyrene particles, labelled with ^{99m}Tc (half-life 13h, isomeric transition, 141 KeV γ), were produced from a spinning disc generator and collected in a liquid impinger. These were resuspended in a 200 l reservoir and administered by mouth breathing via a respiratory valve and tube. The experimental programme was designed to investigate the effect of three parameters, namely particle diameter (2.5 to 7.5 μm), tidal volume (0.5 to 2 l) and breathing rate (10 to 25 per minute). Each subject took part in at least one series of experiments, in which only one parameter varied. All the volunteers exhibited a normal lung function as assessed by a forced expiratory spirogram.

Deposition was assessed in terms of the total amount of material deposited and the relative deposition in different regions of the respiratory tract. Total deposition was estimated from the difference between the concentration multiplied by the volume of aerosol inhaled and the mass of aerosol exhaled. Deposition in the mouth and larynx was estimated from mouth washings immediately following administration. Clearance from the lung was followed for two days after administration using a whole-body monitoring facility, comprising pairs of collimated 15cm NaI(Tl) detectors mounted coaxially above the chest and stomach. Two exponential phases of clearance were observed, the faster being taken to represent mucociliary clearance of material deposited in the tracheobronchial (TB) region of the lung and the slower, the pulmonary (P) region.

Total deposition

Total deposition, expressed as a fraction of the inhaled aerosol, was compared with the predictions of models proposed by the ICRP (1966, 1979) by plotting it against an impaction parameter D^2F (where D is the aerodynamic diameter and F the average inspiratory flow-rate). If impaction is the sole mechanism by which deposition takes place, then this correlation should yield a smooth curve. Initially, the predicted values of D^2F for an experimental group were used (EUR-6766, p723). However, because the inspiratory flow-rate varied between subjects and particle diameter varied slightly from batch to batch, the data were replotted using actual values of D^2F. It then became apparent that the correlation was still unsatisfactory, as consideration of different experimental series showed that impaction is not the sole mechanism of deposition; nevertheless it remains a convenient way to express the data. To improve the statistics, the data were replotted in histogram form

against the logarithm of D^2F (see Figure 1a). This enabled approximately equal numbers of experiments to be considered within each histogram bin. Data appear in agreement with the original ICRP model and are even better matched with a recent revision of this, which is not shown. Values also fall in the middle of the range of data recently reported by various authors.

Regional deposition

Regional deposition data were treated similarly. Values for mouth deposition are in good agreement with previously published work, but tend to be lower than the ICRP predictions. This could be because the aerosol was administered via a mouth tube, rather than under normal inhalation conditions.

It was not possible to express the regional lung deposition data directly as a fraction of the inhaled aerosol. Many problems arose in the calibration of the whole-body monitor including a) material trapped in the oesophagus following clearance and b) material in the gastro-intestinal (GI) tract contributing to the chest radioactivity measurement. A comparison of the total deposition estimated in the usual manner with estimates from whole-body and excretion measurements demonstrated quite large discrepancies, mainly due to problems associated with material in the GI tract. It is suggested that much of this is due to material deposited in the pharyngeal region not removed by mouth washing, but clearing to the stomach in the time between administration and the initial whole-body measurement (about 20 minutes). To reduce these errors, regional deposition in the lung was expressed as the fraction P/(P+TB). As shown in Figure 1b, deposition is also in reasonable agreement with the ICRP predictions, although, in this case, agreement is less good with the more recent revision. These data are reasonably consistent with other published work, although, due to the errors outlined above, greater discrepancies occur than for total deposition.

A comparison of deposition in smokers and non-smokers

As can be seen from the figures, no significant differences in either total or regional deposition were found between the smoking and non-smoking groups, although the smokers did exhibit a greater variability. To assess the usefulness of deposition measurements as a test of lung function, experiments in which at least five non-smokers had participated were used to define the normal range of depositions for a particular inhalation regime. Assuming a Gaussian distribution about the non-smoking mean, a list was compiled of the number of standard deviations by which the data for each subject differed from the mean. Then, using a standard multinomial expression, the probability that a subject would be different from the mean on those occasions purely by chance was calculated. This analysis yielded a significant difference between the smoking and non-smoking groups ($p < .002$) indicating that only non-smokers should be used to define the normal range. There was no correlation between those subjects yielding a result that would occur by chance less than 5% of the time, with either their smoking history or lung function test data. Of the 31 participants, one smoker exhibited results highly different from the normal range ($p < 1 \times 10^{-10}$). Five years after the study, a repeat lung function test showed a drop in his forced vital capacity of 30% of its predicted value compared with a mean drop for the non-smokers over this period of 4.9 ± 2.9 (SD)%. This subject now exhibits clear signs of small airway obstruction. If this type of technique has a potential medical benefit, it will be necessary to establish the normal range of values more precisely and for prospective patients to take part in a number of experiments.

Short-term clearance

Studies of clearance were hampered, in certain instances, by low TB depositions leading to some measurement errors. However the range of half-times for the fast phase (1.1 to 71 h) exceeded the 10 minute half-time proposed by the ICRP in all cases. In the ICRP model, this is followed by clearance from the P region (half-time 24 h) dependent upon recruitable macrophages, before the longer-term alveolar clearance commences. This hypothesis is not supported by our results, since the time taken for the fast phase of clearance to stop was independent of particle size and therefore of the site of the deposition. In addition, the half-time of clearance for 2.5 μm particles was over 6 times that of the 7.5 μm particles. It

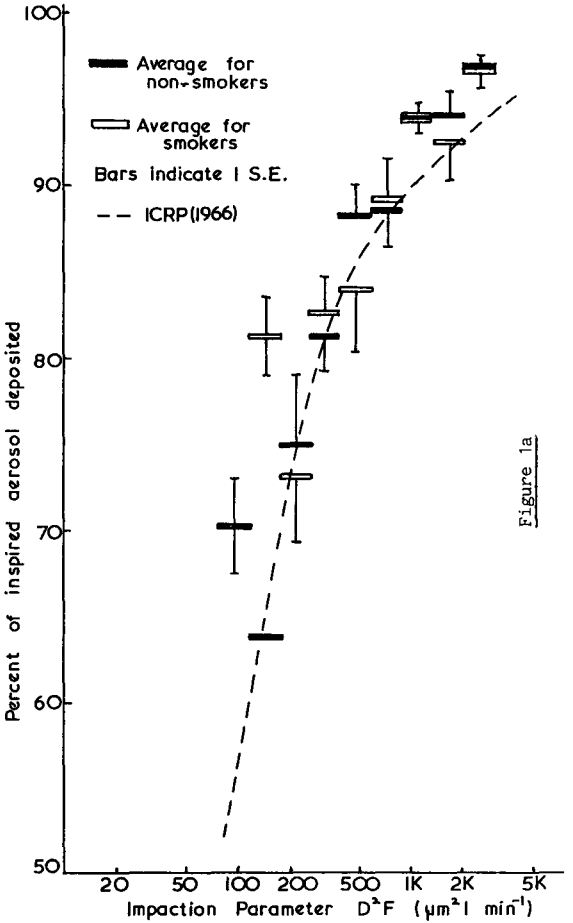

Figure 1a

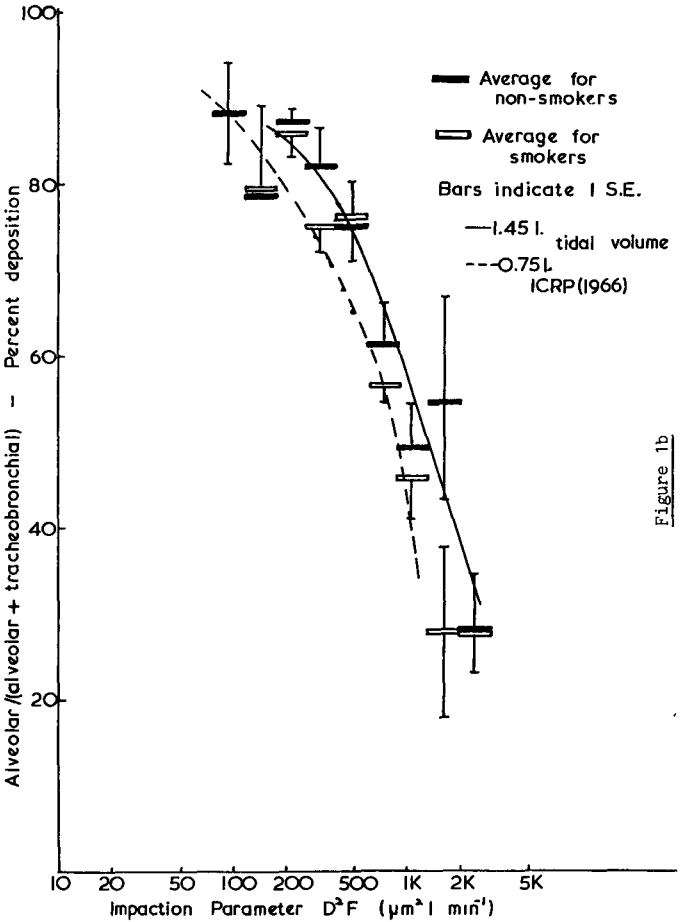

Figure 1b

is difficult to envisage a mechanism involving macrophages which could explain this. It seems more likely that the point at which the fast phase stops represents the clearance of particles deposited at the bottom of the mucociliary escalator. Differences in the clearance half-times probably arise from the sites along the pathway where deposition by impaction occurs preferentially.

Further evidence came from a comparison of smokers with non-smokers. Mean clearance half-times for non-smokers were up to twice those for smokers, with the times for the fast phase to stop correspondingly longer, there being no significant differences in P/(P+TB). Those differences disappeared, however, if the clearance half-times for individual subjects for 2.5 and 5.0 μm particles were expressed in terms of that for 7.5 μm particles. This suggests that cigarette smoking enhances the mucociliary action rather than, as the ICRP suggest, retarding it.

Summary

Deposition data are in good agreement with previously published work, and demonstrate that the ICRP predictions are satisfactory, although their assessment of short-term clearance may need to be reviewed. Cigarette smokers as a group exhibit different deposition patterns to non-smokers, although all but one of the volunteers were within the non-smoking range as individuals. In addition, short-term clearance is considerably enhanced in cigarette smokers. In the future, it may be possible to use deposition techniques as more sensitive indicators of lung function than conventional tests currently available.

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Legend to Figures

Figure 1. A comparison of the data obtained with the predictions of the ICRP model for (a) total deposition and (b) regional lung deposition.

NB Horizontal bars mark the top of the histogram bins.

Contractor: Medical College of St. Bartholomew's Hospital, Department of Radiobiology.

Contract No. : 252-77-1 BIO UK

Head of Research Team : Professor Patricia J. Lindop

General Subject of Contract : Radiation damage from inhaled particles in the rodent lung.

The work carried out in the three projects is designed to assess qualitatively and quantitatively the hazards from inhalation of radioactive particles, with the initial emphasis on plutonium as insoluble particles in the murine lung.

The projects cover the fields of particle size distribution and microdosimetry in the lung; the effects of particle size and dose on the metabolism and translocation of plutonium from the lung, and effects on non-neoplastic, early and late changes, as measured by alveolar macrophages, and fibrosis, as well as neoplasia.

As had been planned the results from each project are mutually interdependent. The Project Directors and scientific personnel involved are therefore listed, and the detailed results from the projects are integrated for the programme as a whole.

Project 1: Head of Project and scientific staff: A. Morgan, A. Black, (AERE, Harwell) S. R. Moores, M. Walsh, J. Pritchard

Title of Project: Kinetics of particulate PuO_2 in the lung.

Project 2: Head of Project and scientific staff: B. E. Lambert, J. Shewell, (SBHMC) K. Danielak, D. M. Peel, J. D. Tarling

Title of Project: Late effects produced in the lung as a consequence of inhalation of plutonium.

Project 3: Head of Project and scientific staff: J. E. Coggle, J. Shewell, (SBHMC) J. D. Tarling, D. M. Peel

Title of Project: Early effects of particulate PuO_2 (and other internal emitters) on the lung.

Introduction

During the period under review a glove-box suite for the exposure of mice to aerosols of sized $^{239}PuO_2$ was constructed (Walsh et al., 1978; 1980). It was commissioned using neutron irradiated thorium oxide (ThO_2) as a convenient analogue for $^{239}PuO_2$ (Black et al., 1978; 1979; Moores et al., in press). In addition, this work provided information on the regional deposition of particles with a similar density to $^{239}PuO_2$ and on the levels and persistence of pelt contamination.

Following the commissioning of the suite, 'nose only' exposures have been carried out of male and female SAS/4 mice, aged 6 weeks, to sized $^{239}PuO_2$ particles with activity median aerodynamic diameters (AMADs) of 0.8, 1.5 and 2.2 μm (subsequently referred to as small, medium and large respectively). The range of initial alveolar deposition (IADs) from 0.1 to 25 nCi (4 to 924 Bq) was selected as it appeared to give maximum lung tumour incidences whilst avoiding mortality from early pneumonitis. Details of the target IADs and those actually achieved in the groups of mice exposed for late-effect studies are given in Table 1. Additional groups of mice were exposed as indicated in Table 1 for studies of the fibrogenic effects of $^{239}PuO_2$ and to investigate its retention/metabolism for dosimetry purposes. The numbers of mice exposed for studies of late-effects, fibrosis and retention/metabolism are about 1800, 330 and 1050 respectively, exclusive of controls.

Table 1. Details of administration of sized $^{239}PuO_2$ to mice for studies of late-effects, fibrosis and retention/metabolism.

Target IAD nCi	Bq	IAD achieved, nCi (Mean ± S.E.) AMAD 0.8 μm	AMAD 1.5 μm	AMAD 2.2 μm
0.1	4	0.13 ± 0.01**	0.12 ± 0.01*	0.11 ± 0.01**
0.5	19	-	0.74 ± 0.05	-
1.0	37	-	1.31 ± 0.06	-
2.0	74	2.66 ± 0.10**	3.27 ± 0.22*	2.10 ± 0.11**
5.0	185	-	4.40 ± 0.22	-
10.0	370	11.24 ± 0.47	9.53 ± 0.50	12.71 ± 0.65
25.0	925	-	26.54 ± 2.44*	40.38 ± 3.70**

* Includes groups exposed for retention/metabolism studies
** Includes groups exposed for retention/metabolism and fibrosis studies

Deposition of sized $^{239}PuO_2$

The alveolar deposition of sized $^{239}PuO_2$ has been described by Morgan et al., (in preparation). In each exposure, measurements were made of the mean concentration of airborne $^{239}PuO_2$ and its AMAD. The IAD was determined by killing a number of mice 2 days following exposure, by which time muco-ciliary clearance of particles deposited in the conducting airways was virtually complete. In Fig. 1, the normalised alveolar deposition of sized $^{239}PuO_2$ is plotted against AMAD. Corresponding data for ThO_2 are included. It is apparent that the AMAD corresponding to maximum alveolar deposition in

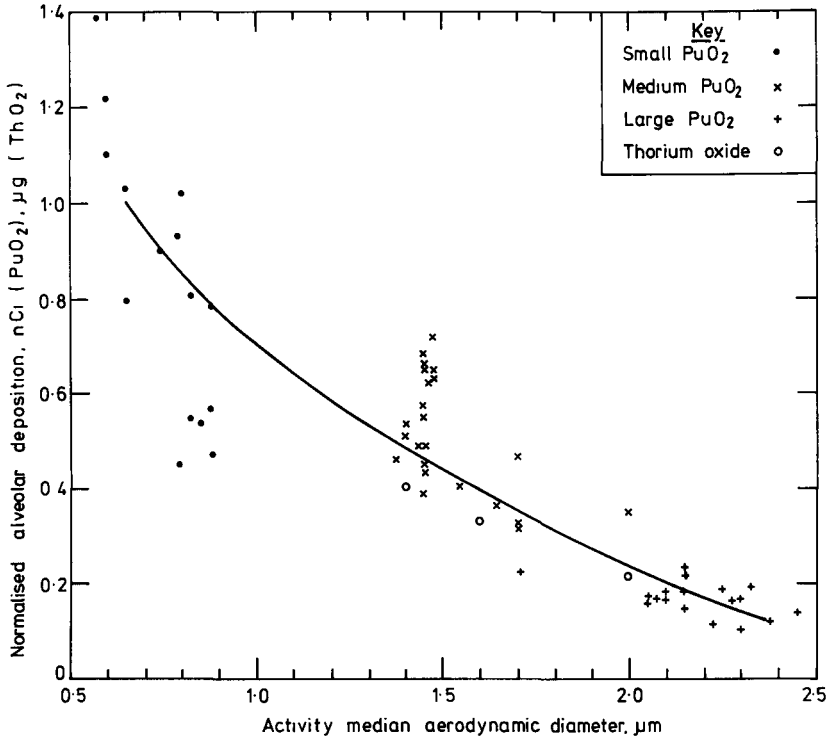

Fig. 1. Alveolar deposition of sized $^{239}PuO_2$ normalised to an aerosol dosage of 100 nCi l^{-1} min. and of sized ThO_2 normalised to an aerosol dosage of 100 µc l^{-1} min.

CLEARANCE OF ^{239}Pu FROM THE MOUSE LUNG AFTER INHALATION OF $^{239}PuO_2$ PARTICLES (1.5 µm AMAD)

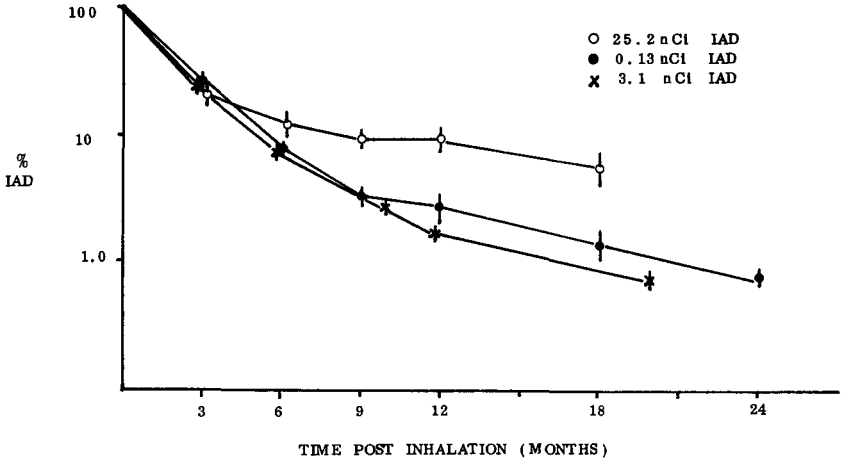

Fig. 2 Clearance of ^{239}Pu from the mouse lung after inhalation of $^{239}PuO_2$ particles (1.5 µm AMAD).

the mouse is less than the value of 2 μm reported for the rat.

Relative lobar concentrations (RLCs) were measured 2 days after exposure. The accurate quantification of variations in RLC is of considerable importance in assessing the lung dosimetry of inhaled $^{239}PuO_2$. As observed in the rat and hamster, the highest RLCs were found in the right apical lobe.

Retention/metabolism studies

In this investigation, mice were killed at 6 time points up to 2 years after administration of $^{239}PuO_2$ to determine the retention of ^{239}Pu in the lung and its translocation to other organs. These studies are necessary to enable average lung doses to be determined. As shown in Fig. 2, it is clear that retention is affected by the amount of ^{239}Pu deposited but there does not appear to be any marked dependence upon particle size. Thus, for IADs of 0.1 and 3 nCi the lung retention of ^{239}Pu could be described by a two-component exponential expression with half-times of about 50 and 210 days. However, in mice with an IAD of 25 nCi the fraction retained with a half-time of 50 days was smaller and the half-time of the second component much longer ($\sim$ 420 days).

Differences in the retention of ^{239}Pu in the lung correlate well with measurements of ^{239}Pu in faeces. As shown in Fig. 3, for example, for IADs of 0.1 and 3 nCi faecal excretion rates were approximately constant during the first year at about 1% of the contemporary lung content per day. However, for mice with an IAD of 25 nCi there was a fall in faecal excretion rate over a period of 6-9 months to a value which is over an order of magnitude lower.

Significant translocation of ^{239}Pu to other organs was observed. At 18 months after administration the fractions of the IAD found in the cervical lymph nodes and in liver were 0.15 and 0.03% respectively compared with 2-5% of the IAD in the lungs at that time. Small, but significant, amounts of ^{239}Pu were also detected in bone. Because of the constancy of the faecal excretion and the relatively low uptake into other organs it is evident that most of the Pu removed from the lung is mobilised by the macrophage-muco-ciliary escalator-gut route even at long times after inhalation.

Short-term effects of $^{239}PuO_2$

Studies have been made of the short-term somatic effects of inhaled $^{239}PuO_2$ on the mouse lung. In particular, the effect on the alveolar macrophage (AM) population of the lung has been studied using an *in situ* bronchopulmonary lavage technique to recover a proportion of these free cells *post mortem*. In addition to the number of AM recovered, the associated ^{239}Pu activity was determined, together with that remaining in the lung. If

it is assumed that the AM recovered are representative of those in the alveolar spaces and also, that there has been no transfer of ^{239}Pu to the interstitium or pulmonary lymphatics, it is possible to estimate the total number of free AM. Some control animals were exposed to neutron-irradiated ThO_2 and the induced ^{233}Pa gamma-activity used to estimate the total number of free AM (about 3×10^6) in these animals.

Changes in the total numbers of AM in the lung following the administration of 17 nCi (630 Bq) of medium sized $^{239}PuO_2$ are shown in Fig. 4. There was a decline in number over a period of about 2 weeks, followed by a gradual increase up to 2 months. It was found that the deposition of as little as 0.5 nCi (19 Bq) produced a significant depression in AM numbers. Following the alveolar deposition of more than about 2 nCi (74 Bq) there was a chronic depression in AM numbers that persisted until the end of the experiment (4 months). Referring to Fig. 4, the magnitude of both the acute (A) and chronic (B) depression in AM numbers was dose dependent, as was the time taken (Z) to reach the minimum and the time taken (Y) to recover to a quasi-equilibrium. There was an increase in the diameter of AM recovered from $^{239}PuO_2$ exposed mice and the cells had a reduced viability as measured by the exclusion of trypan blue.

Late non-neoplastic effects of $^{239}PuO_2$

The fibrogenic potential of $^{239}PuO_2$ is a non-neoplastic end-point under long-term investigation. As indicated in Table 1, groups of mice have been exposed to the small and large sized $^{239}PuO_2$ to give IADs of about 0.1, 2 and 25 nCi. Data are available for these experiments using large sized $^{239}PuO_2$ but those involving the small are incomplete.

Measurements of lung weight, DNA, protein and collagen (Moores et al., 1979) are being made on pooled samples of individual lobes. Considerable development work has been required to adapt analytical procedures to the very small amounts of tissue available. As the levels of proteoglycans in mouse lung have proved to be less than half that in the rat it has not been possible to include their assay on the lobar samples. However, separate determinations of the proteoglycan-constituents, hexuronic acid and hexosamine will be carried out on pooled lung samples.

Some data on the fresh lung weights of mice exposed to the large sized $^{239}PuO_2$ are given in Table 2. A dose-related increase in lung weight was apparent, especially when expressed as a proportion of body weight. There was a significant increase in the variance of the populations with IAD, probably attributable to subpopulations of grossly affected individual

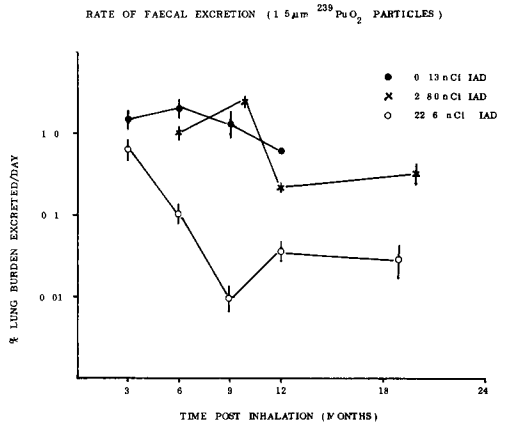

Fig. 3 Rate of faecal excretion (1.5 µm $^{239}PuO_2$ particles).

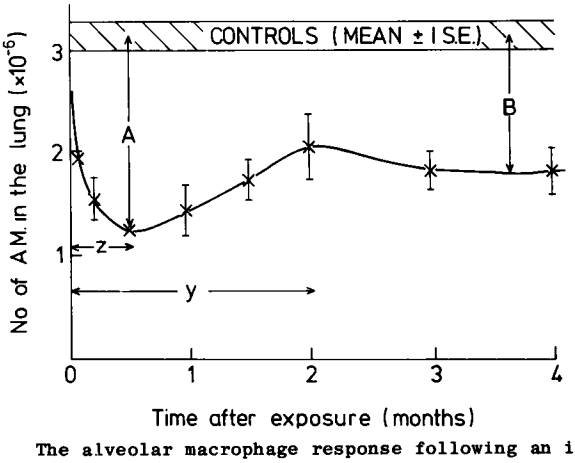

Fig. 4 The alveolar macrophage response following an initial alveolar deposition of 1 nCi of $^{239}PuO_2$.

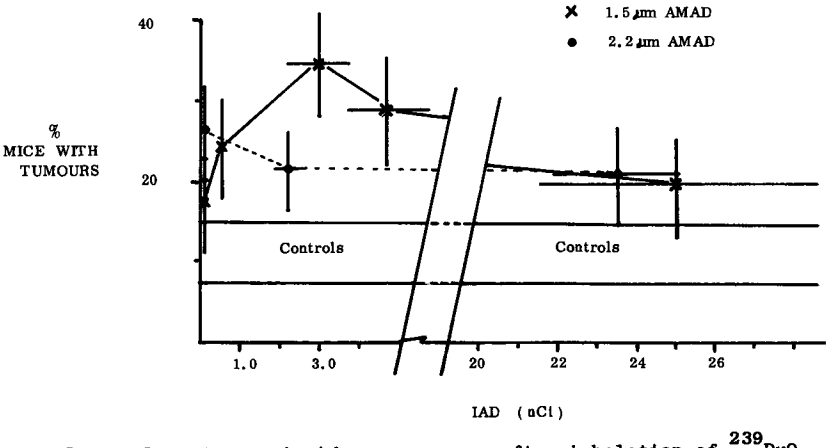

Fig. 5 Lung tumour incidence one year after inhalation of $^{239}PuO_2$ particles.

animals. The increase in lung weight after exposure to PuO_2 was accompanied by an increase in the hydration of tissue associated with cellular consolidation and oedema observed in separate histopathological investigations. Preliminary results from the biochemical determinations indicate that most of the increase in dry weight was due to protein, although its concentration in the lung remained relatively constant. Similarly, the concentration of collagen did not increase with IAD; it even declined in some cases. However, the total collagen content of the lung increased by over 100% in many mice in the high dose group.

Table 2. Lung weights of mice 18 months following exposure to large sized $^{239}PuO_2$ (Mean ± S.E.)

Exposure group	No. of mice	Lung weight (mg)	Lung weight (% of body weight)
Unexposed controls	9	185 ± 10	0.54 ± 0.02
ThO_2-exposed controls	12	195 ± 10	0.49 ± 0.02
0.15 nCi IAD	9	210 ± 23	0.58 ± 0.04
1.10 nCi IAD	13	245 ± 34	0.70 ± 0.10
25.00 nCi IAD*	6	350 ± 52	1.10 ± 0.24

* Killed at 16 months

The distribution of fibrotic changes in the lung lobes did not appear to reflect the initial RLCs. For example, it might have been expected that the right apical lobes (highest RLC) would have shown the greatest fibrotic changes. In fact, the right diaphragmatic lobes (lowest RLC) showed the greatest increase in dry weight. More detailed analysis of the lobar distribution of fibrotic lesions are being undertaken in parallel histological studies.

Long-term studies (neoplasia and survival)

For long-term studies mice were divided into five groups: (i) mice killed at one year post inhalation to score the numbers as well as the morphological classification of lung tumours using (initially) a histological clearing technique; (ii) mice killed when moribund. These were subject to a full histopathological investigation. Each mouse dying in all groups has been subject to the same detail of histopathological examination.

The incidence of lung pathology at 1 year and general pathology and survival have been assessed in the 1.5 (medium) and 2.2 μm (large) size groups at present.

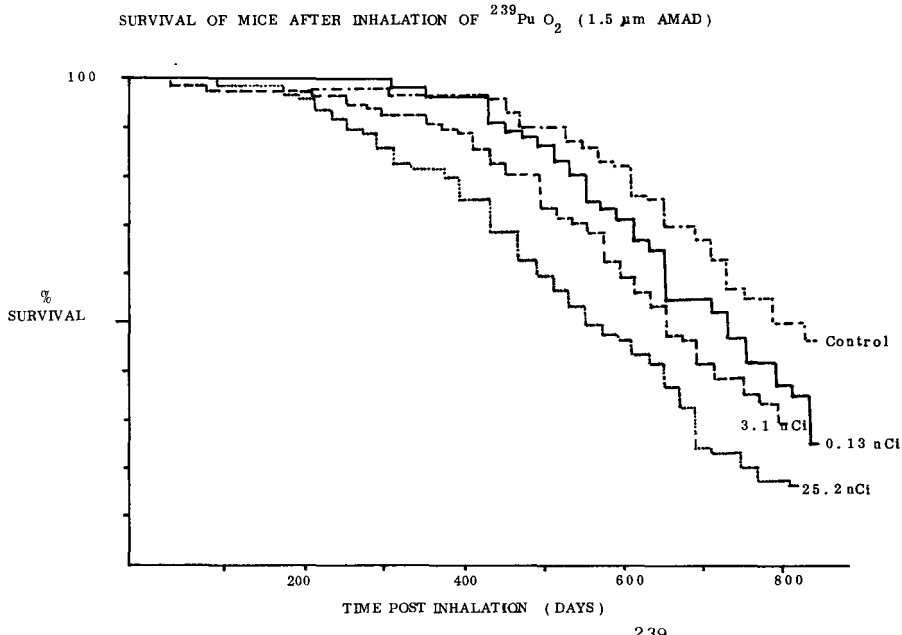

Fig. 6 Survival of mice after inhalation of $^{239}PuO_2$ (1 [illegible] µm AMAD)

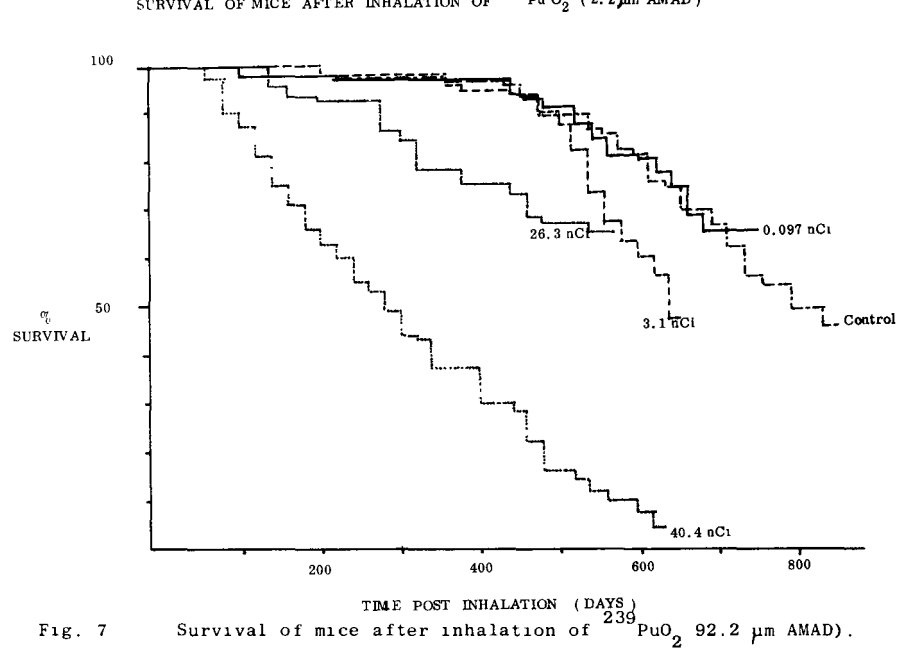

Fig. 7 Survival of mice after inhalation of $^{239}PuO_2$ 92.2 µm AMAD).

The incidence of lung tumours at 1 year after inhalation in these groups is shown in Fig. 5 - these should be compared with spontaneous incidence in control groups which was 10.8 ± 3.6% (S.E.). The more complete data for the 1.5 μm particle size groups indicate a peak incidence of tumours (34.8 ± 7.0%) in mice with an IAD of 3 nCi. Fig. 5 also indicates a possible difference in peak tumour incidence between the medium and large size particle groups - until more complete data are available it is difficult to judge whether this difference is significant. It is clear that at higher IADs the incidence of tumours fell but overall survival was also greatly reduced (see Figs. 6 and 7), due mainly to radiation induced pulmonary insufficiency. All tumours seen in these mice were adenomas or adenocarcinomas (in approximately equal numbers). By far the greater proportion of these tumours originated in the alveolar region of the lung as judged by their site and cell type (cuboidal epithelial), and very few were composed of bronchiolar (columnar) cells only. There was no correlation between lung lobe size (and therefore numbers of cells at risk) and tumour incidence.

A striking feature of the histopathology of lungs of mice killed at 1 year in the higher IAD groups, and all the lungs at longer times post inhalation was the accumulation of giant macrophages some of which filled the alveolar spaces. These were often associated with areas of fibrosis and alveolar cell hyperplasia which obliterated the normal lung architecture.

It is clear therefore that at lower IADs radiation damage to the lung was expressed chiefly in a higher incidence of tumours whereas at the highest IADs in this experiment (> 20 nCi), mice died early because of lung failure due to fibrosis and cell accretions, etc. However, even at the lowest IADs overall survival was significantly decreased as compared with sham exposed controls (Figs. 6 and 7). The numbers of mice exposed to the 0.8 μm and 2.2 μm particles which have to date died are insufficient to make a firm assessment of the effect of particle size on long term survival but the trends indicate no significant differences.

Full histopathological studies are being carried out on all mice that die and so far there is evidence of a great increase in lung pathology at death particularly in the highest IAD groups - death in these groups occurring so early that leukaemia is of no significance as a cause of death, whereas at intermediate IADs (e.g. 3.4 nCi) leukaemia is slightly increased (by about one-third).

Microdosimetry studies of $^{239}PuO_2$ in the lung

To calculate the dose to different regions of the lungs in mice and

to relate it to studies of late-effects, it is necessary to determine (a) the size distribution of particles of $^{239}PuO_2$ in the alveolar region (b) the distribution of particles within the lung, and (c) any changes in particle size and distribution which may occur over the life-time of the animals. A stripping-film autoradiographic procedure is being developed to provide information on these factors.

Lung from exposed mice have been fixed by various methods, embedded in either wax or plastic, and sections cut at 2 to 5 μm in thickness. In addition to contact autoradiographs, in some cases 10 and 15 μm transparent spacers were interposed between section and emulsion. The autoradiographic efficiency of track production is currently being assessed by comparing the total number of tracks detected with the ^{239}Pu activity of the corresponding section, determined by radiochemical analysis. The highest efficiencies are obtained with a 10 μm spacer, because, in contact autoradiographs, the tracks at the centres of the large 'stars' are too dense to be resolved. With a spacer, the tracks are shorter and can be resoved more readily.

To determine particle size distribution in full, it will be necessary to prepare autoradiographs exposed for a range of times sufficient to allow the smallest particle present to produce 3 or 4 tracks. Under these conditions, 'stars' produced from larger particles are too dense to count. Before any information on the distribution of particles in the lung can be obtained, it will be necessary to establish that this is not affected by the procedures used for lung fixation and sectioning.

To facilitate this work the possibility of using an image analysis system (Quantimet 720, Cambridge Instruments Ltd.) for track counting is being investigated.

Conclusions

Although this work is, as yet, incomplete the general objectives are nearly achieved. The apparatus and techniques necessary for the production of three sized aerosols of $^{239}PuO_2$ have been developed and applied to the exposure of large numbers of mice. The resulting effects of this exposure have been assessed in both the short- and long-term with a variety of end-points enabling dose (1AD)-response curves to be drawn. However, whereas the effects of varying amounts of plutonium deposited in the lung are clear the evidence for the influence of inhaled particle size is equivocal. Data from the mice exposed to the small (0.8 μm AMAD) aerosol particles, soon available, will make more positive conclusions possible.

The direct extrapolation of these results to man may be difficult

owing to the suggested differing long-term response of human lung and mouse lung to plutonium. However, as a model system the mouse lung should provide data from which risk estimates for man may be made.

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Contractor : The Polytechnic of Central London, UK
Contract nr : 243-77-1 BIO UK
Head of Research Team : Dr. J. A. Simmons

General subject of contract : Microdosimetry of Lung

Head of Project and scientific staff :

Dr. J. A. Simmons
Mr. C. Sims (April 1977 - September 1978)
Mr. S. R. Richards (February 1979 -

Fears have been expressed that, if a radioactive particle is inhaled into the lung the particles which it emits (e.g. α's or β's) may give rise to a considerable amount of damage in a small volume. In order to consider this possibility in a rigorous manner it was believed desirable to estimate the dose delivered to the irradiated material, and this study originally attempted to calculate the dose to lung tissue surrounding an inhaled $^{239}PuO_2$ particle. It soon became apparent,however, that "dose" may not be the most appropriate parameter to measure; instead, "specific energy" appeared to be a better alternative. This allows one to calculate the relative energy depositions in elemental volumes of material actually traversed by the alpha-particles.

All measurements described in this report have been made on rat lung. Sections of lung were fixed at 10 cm inflation pressure and stained so that the air in the alveolar sacs appeared light against a dark grey background of tissue when viewed on the screen of an image analyser known as the Magiscan. This machine functions by viewing the material to be analysed with a television camera attached to a microscope. The signal obtained is digitised by a high-speed a/d converter to create a raster scan of the image. These elements are stored in a dedicated mini-computer in such a way that the specific locations of air and tissue are noted as 0's and 1's. The picture is re-created for display on a viewing screen, and by means of a light pen a site was chosen at some particular point on the surface of an alveolar sac where it was assumed that a $^{239}PuO_2$ particle had been trapped. The hypothetical α-particles emitted from it were then followed through tissue and air. The range of each particle was then caclulated using energy-loss formulae appropriate to α-particles with an initial energy of 5.1 MeV. For simplicity, it was assumed that the energy deposition events occurred at distances of 0.1 μm separation and that the energy loss in air was small enough to be ignored. Fifty alpha tracks at equal angular separations were followed, and this was repeated for four different sites.

In order to calculate a specific energy it was necessary to define a volume in which one was considering the energy deposition. This was done by first constructing a series of concentric rings with the plutonium particle at the centre and extending to the maximum range of the α-track. Since the calculations were for 0.1 μm increments of distance, this was the width chosen for each ring. As explained above 50 tracks at equal angular separations were generated and accordingly each annulus was divided into fifty segments. The volume, and hence the mass, of each segment was then taken as appropriate. This process was continued until there were no further α-tracks in the region considered.

The simplest calculation which can be performed is simply to sum all the volumes derived as above to give the maximum volume of tissue which can be irradiated. Results for the four sites investigated are shown in Table 1, together with results derived by scaling to the human lung.

Table 1

Volume of lung irradiated by one $^{239}PuO_2$ particle (in mm^3)

Site	Rat	Man
1	.0051	.013
2	.0292	.113
3	.0170	.067
4	.0250	.097

The assumptions made in extroplating from the rat to the human case are:-

1. The structure of rat and human lung is fundamentally similar.
2. The human lung is a scaled-up version of the rat lung, the human alveoli being about 3 times bigger in diameter.
3. The ratio of tissue volume to total parenchymal lung is 0.1 during normal human respiration.

The volumes of human lung which would be irradiated by the maximum permissible lung burden of 16 nCi depends, of course, on the numbers of particles involved and hence the size of each particle. Table 2 shows results assuming that all have a true diameter of 1 μm.

Table 2.

Volume of human lung irradiated by 16 nCi of 1 μm particles

Site	Volume (cm^3)
1	0.63
2	5.49
3	3.26
4	4.71

Two other sets of calculations of greater complexity can also be carried out on the basis of the measurements described above. The first gives a histogram of the specific energy as a function of distance from the plutonium dioxide particle. This is obtained by summing all the volume elements and all the energy depositions from all 50 tracks for each 10 μm interval of range. The energy and volume associated with each of these intervals are then converted to specific energy. In calculating the energy deposited it has been assumed that the 50 tracks simulated are representative of a much larger number; the number actually taken (9500) are those which would have been emitted from a particle of 0.03 p Ci activity during a period of 100 days. (A span of 100 days was selected to correspond approximately to the mean life of parenchymal lung cells). Results for rat and simulated human lung are shown in Figure 1 from which it can be seen that there is very little difference between the two cases. It can also be seen that, in the human case, specific energies of the order of a rad can be deposited at distances of up to 1500 μm (i.e. 1.5 mm) from the particle. Whether such small specific energies can cause any definite effect has yet to be ascertained.

The other set of calculations referred to above are those which give histograms of volumes v. specific energy. These were obtained by summing all the volumes receiving a specific energy within a specific energy interval. According to the activities considered, intervals of 0.1 rad/100 days, 1.0 rad/100 days and 10 rad/100 days were selected. Results from one site (which is believed to be typical) are shown in Figure 2. These, in common with the results from the other three sites, show that a much greater volume of human lung receives very low specific energies than does rat lung. Also, as a general trend, the rat volumes receiving a particular specific energy is somewhat greater than the human volumes. Due to the variability of the lung structure, however, the details of the four plots have little else in common.

Although the calculations of irradiated volumes, together with the distributions described above, are clearly of importance it was felt that events at a cellular level could possibly be of even greater significance. In order to investigate these in detail two assumptions, both based on the work of Weibel, were made. The first was that 50% of the parenchymal region is made up of lung cells (as opposed to capillary blood or interstitial fluid); the second was that the cells have a volume of 1000 μm^3 and the shape of a flattened ellipsoid approximately 3 μm thick. The exact figure is not very important at this stage as it is the mean track length through a cell which provides the basis of the calculations.

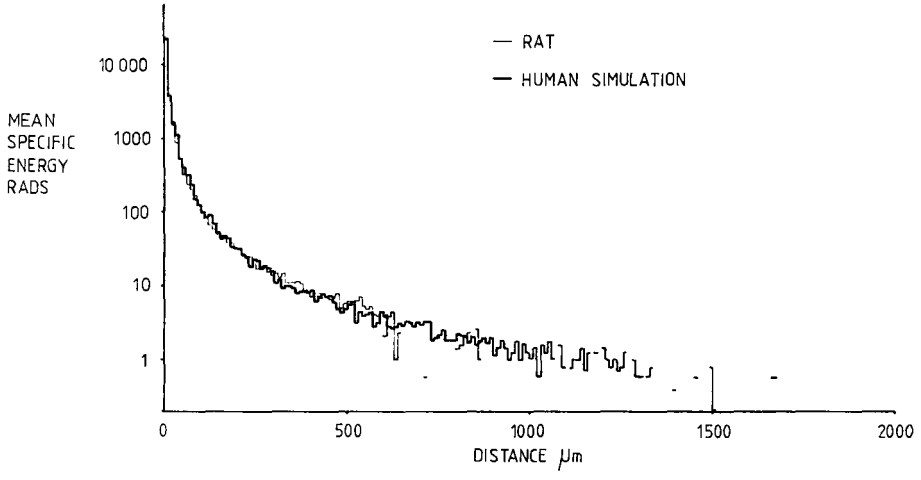

Fig. 1 Histogram of Specific Energy as a Function of Distance (Average of all four sites).

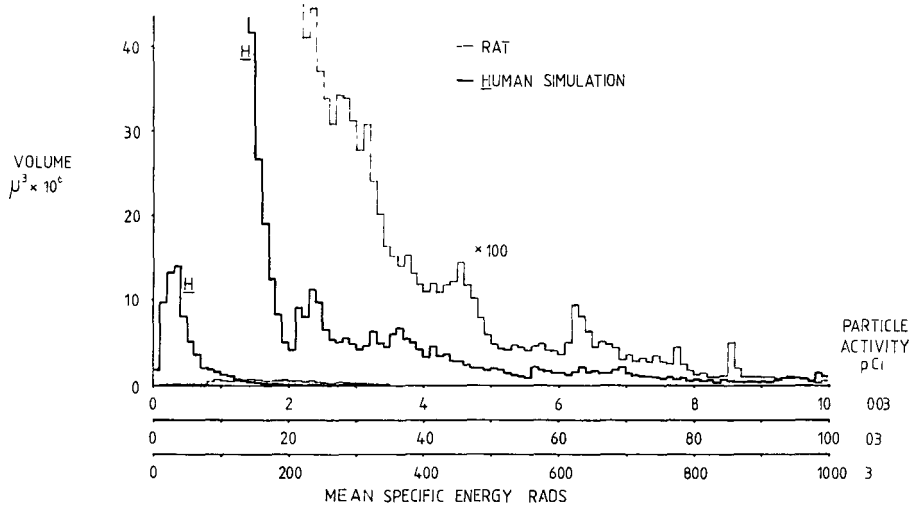

Fig. 2 Histogram of Volume as a Function of Specific Energy (Site No.3)

This mean track length was used to estimate how many cell traversals occur when an alpha particle travels through a layer of tissue.

On this basis, a series of histograms showing the number of cells hit per 10 μm increment of range as a function of distance from the plutonium dioxide particle have been prepared. Also included are the numbers of cells which would be expected to be killed on the assumption of a single, negative exponential survival curve with a D_o of 100 rad. These histograms represent conditions in two different sites for three different plutonium particle sizes. All show very clearly that there can be a large number of cells which are hit but not killed; the signifcance of this is now under investigations.

If the total number of cells hit in a single site is found this can be plotted as a single point on a graph of the number of cells hit as a function of $^{239}PuO_2$ particle activity. Figure 3 shows the curves for the four sites investigated for particles in the range of sizes 0.1 μm → 1.0 μm (activity range .00033 → .33 p Ci). The flattening of these curves at large particle sizes is due to the approach to the limiting condition where all the accessible cells are being hit.

The final parameters of interest here are the numbers of cells hit and killed following retention of the maximum permissible lung burden (16n Ci). In order to calculate these, the numbers of cells involved in one site was simply multiplied by the numbers of particles of a given size contained in the MPLB. This was repeated throughout the range of particle sizes of interest, and the data from all four sites averaged to give a single curve. The results are shown in Figure 4 which also shows the fraction of parenchymal cells irradiated. It can be seen that a relatively small fraction of these cells are hit and, at worst, only 1% are killed.

From this report it is clear that the original objectives of the research programme have been fully met. By moving from concepts of "dose" to those of "specific energy" much more information has been generated than had previously been thought possible, and, in view of this, the work is being expanded to include direct measurements on beagle dog and human lung. Investigations are also under way to assess the effects of very low doses of alpha radiation on cells in culture. All this information, when taken together, should allow much more realistic assessments of maximum permissible lung burdens to be made than is the case at present.

Reports of various aspects of this work have been given at the Fifth International Conference on Medical Physics, Jerusalem, 1979, and the Seventh Symposium on Microdosimetry, Oxford, 1980.

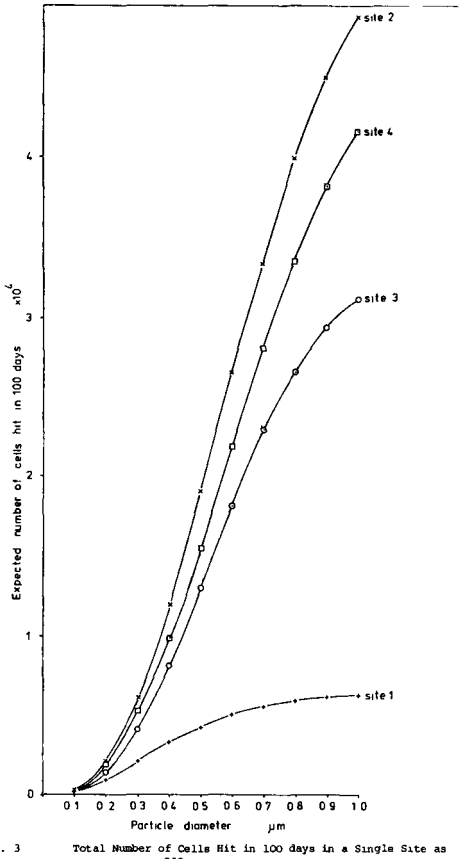

Fig. 3 Total Number of Cells Hit in 100 days in a Single Site as a Function of $^{239}PuO_2$ Particle Size.

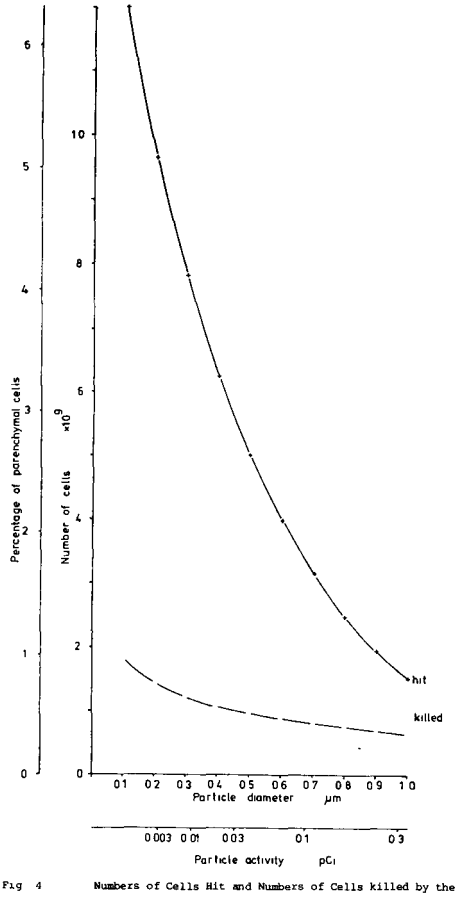

Fig 4 Numbers of Cells Hit and Numbers of Cells killed by the MPLB in 100 days.

Contractor : Medical Research Council, UK

Contract nr : 249-77-1 BIO UK
Head of the research team : Dr J Vennart, Medical Research Council, Radiobiology Unit.

General subject of contract :

Application to radiobiological problems of automated techniques for recognition of visual patterns.

Title of project nr. 1

Dosimetry and effects of radiation distributed inhomogeneously at the cellular level.

Head of Project and scientific staff : Dr L M Cobb (Head)
Mr D Green
Mr G R Howells
Dr M C Thorne

This project has concentrated on the microdistribution and retention of ^{239}Pu in skeletal and other tissues of the female mouse. The distribution studies are intended to provide an adequate basis for ^{239}Pu dosimetry assessment in the female CBA mouse skeleton. The dosimetry studies are necessary to complement ^{239}Pu tumour induction experiments in mice. The programme also includes a comprehensive analysis of the morphological structure of selected bones of the female CBA mouse skeleton, since changes in morphology under pathological conditions, or following exposure to toxic materials, can be useful indicators of the cause of the pathological state or mode of action of the toxin. The initial requirement of the project was to develop α-imaging techniques using solid state fission track detectors for determining the distribution of ^{239}Pu relative to the periosteal and endosteal surfaces of bones with an accuracy of ± 2 μm. Such an accuracy is necessary because alpha-particles emitted by ^{239}Pu have a range of less than 30 μm in mineral bone. Thus, to understand its toxicological effect, it is necessary to have a quantitative measure of distribution with a resolution considerably less than the range of the alpha-particle.

Subsequently the neutron-induced imaging was supplemented by the development of an alpha-radiography imaging technique because of an inability to induce satisfactory soft-tissue images by the neutron irradiation method. Further, the neutron-induced imaging method for the determination of ^{239}Pu about bone surface, which has now been in use for some years, has the disadvantage of being time-consuming. Thus a semi-automated procedure which uses a Quantimet 720 Image Analysis has been developed.

The principal objectives of the project have in general been achieved with respect to investigating, in the CBA mouse skeleton, the spatial distriution of ^{239}Pu about the periosteal and endosteal surfaces as has the detailed morphological analysis of selected bones.

The morphometry was restricted to the ilium, femur, 3rd lumbar vertebra and a central caudal vertebra of the CBA mouse. The results demonstrate considerable variability in many of the morphometric parameters. Special attention has been paid to the degree of anistropy within the bones under investigation since such measurements are necessary for the accurate measurement of depth of burial of plutonium in bone.

The main microdistribution studies have been concentrated on the femur, ilium and lumbar vertebra of the CBA skeleton after intravenous injection of 50 nCi $^{239}Pu\ kg^{-1}$ body mass. At 24 hours after injection the ^{239}Pu distributions are strongly peaked about bone surfaces, with approximately four times more plutonium associated with the endosteal surface than with the periosteal surface. In all features except absolute magnitude, periosteal distributions resemble endosteal distributions, and distributions from cortical bone resemble distributions from trabecular bone. However, although the distribution of ^{239}Pu on various bone surfaces appears to be similar, the absolute magnitude of deposition can vary from one bone to another. It would seem reasonable to assume that ^{239}Pu deposition in bone is influenced more by intrinsic features such as blood supply than by the particular nature of the bone surface.

Subsequent ^{239}Pu distribution studies in the CBA mouse ilium at 10 days, 1, 3, 6 and 12 months after injection show that the peaking of plutonium about the endosteal and periosteal surfaces seen at 24 hours is still evident at 10 days and 1 month after injection, but increasingly more of the ^{239}Pu is buried within the bone cortex. By 3 months after injection a ^{239}Pu peak is still associated with the periosteal surface, but the endosteal peak has mostly disappeared. At 6 and 12 months after injection the distribution peaks associated with both surfaces are no longer evident; the plutonium is randomly distributed throughout the whole bone.

The ^{239}Pu studies in the ilium included an analysis of the distribution of the radionuclide about endosteal surfaces in different regions of the bone; i.e. from the highly trabeculated region close to the epiphyseal plate through to the shaft of the bone where few trabeculae are observed. Such an analysis demonstrated that moving from the trabeculated region through to the cortical shaft, more of the plutonium is associated with

bone substance than with included marrow. Notably, 3 months after injection there is a uniform transition from 63 per cent of the ^{239}Pu being associated with the marrow in the highly trabeculated epiphyseal region of the bone, to only 23 per cent associated with the marrow in the almost untrabeculated shaft.

In an attempt to compare studies involving different levels of administered radionuclide, the distribution of ^{239}Pu about endosteal surfaces of the ilium at different times after injection of 50 nCi kg^{-1} or 1 μCi kg^{-1} were investigated. It was found that the fraction of radionuclide associated with the marrow at 1 and 3 months is largest at the high injection level; the difference being most marked by the later time. Further, at 3 months after injection the ^{239}Pu retention is greatest in ilia at the higher injection level.

Since osteosarcomas are thought to be derived from cells close to bone surfaces, the changes with time of the ^{239}Pu distribution in bone is important for radiation dosimetry purposes. This arises because only ^{239}Pu deposits buried less than 24 μm below bone surfaces irradiate those surfaces or the bone marrow to any significant extent. Further, although the marrow is relatively free from plutonium at early-times after injection, a significant fraction of the marrow close to a bone surface is within range of the plutonium alpha-particles. The change in plutonium distribution seen at 3 months or later after the injection of the radionuclide, which produces a build-up of plutonium in the marrow, results in a change in the fraction of the marrow being irradiated from that irradiated at the earlier time where the plutonium distribution is still peaked about bone surfaces. Such observations have important implications in relation to ^{239}Pu radiation dosimetry when applied to the possible induction of marrow tumours.

Publications resulting from this work

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Thorne, M.C., Nature, Lond. 259, 539-541, 1976.

Green, D., Howells, G.R., Thorne, M.C. and Watts, R.H., Int. J. Appl. Radiat. Biol., 29, 285-295, 1978.

Green, D., Howells, G.R., and Thorne, M.C., submitted to Phys. Med. Biol., November 1980.

Green, D., Howells, G.R., and Thorne, M.C., Proc. IVth ICPA Congress, Paris Vol. 2, 405-512, 1977.

Green, D., Howells, G.R. and Thorne, M.C., Int. J. Radiat. Biol., No. 34, 1, 27-36, 1978.

Green, D., Howells, G.R. and Thorne, M.C., Int. J. Radiat. Biol., No. 5, 36, 499-511, 1979.

Howells, G.R. and Green, D., 15th Ann. Meeting of the European Society for Radiation Biology and the Symposium of the European Late Effects Project Group, Rotterdam, 25-29 August 1980.

Green, D., Howells, G.R. and Thorne, M.C., J. Micros., 122, Pt. 1, 1981.

Thorne, M.C., Phys. Med. Biol., 22, No. 1, 36-46, 1977.

Contractor : Medical Research Council, UK

Contract nr : 267-78-1 BIO UK
Head of the research team(s) : Dr J Vennart, Medical Research Council, Radiobiology Unit.

General subject of contract :

Behaviour and carcinogenic effects of radioactive particles in the respiratory system.

Title of the project nr. 1

Behaviour and carcinogenic effects of radioactive particles in the respiratory system.

Head of Project and scientific staff : Dr L M Cobb (Head)
Dr A L Batchelor
Dr D J Gore
Mr T J Jenner
Dr G Patrick

This project is in 2 parts. The first is the BEHAVIOUR and the second the CARCINOGENIC EFFECTS of radioactive particles in the respiratory system.

Part I - BEHAVIOUR

The results and their relevance for radiation protection

1. It had been discovered that when insoluble particles of $^{133}BaSO_4$ were deposited on the surface of the distal trachea of the conscious rat, rapid muco-ciliary clearance did not remove all the particles; in particular about 1% remained associated with the wall for at least 30 d. This was at variance with the dosimetric model for insoluble radioactive particles adopted by the International Commission on Radiological Protection (ICRP), in which the airways are rapidly cleared. Since it is thought that the dose to the bronchial epithelium may be important in determining the risk of lung cancer in man, experiments were devised to quantify particle retention in the airway wall, and to study the mechanism involved.

2. Following deposition in the distal trachea of the rat via an intratracheal cannula, other particles showed similar retention to $^{133}BaSO_4$. For 1.1 µm count median diameter particles of fused aluminosilicate, retention after 7 d was 0.45%, and for 5.7 µm diameter particles it was 0.75%. With 0.35 µm diameter particles of $^{235}UO_2$, injected in the same way, 0.69% was retained after 14 d. Thus particles of different materials and diameters were retained to a similar extent. The mass of inhaled $^{235}UO_2$ (count median diameter 0.32 µm) in the trachea and extrapulmonary bronchi including the first bifurcation was also determined. The amount on the trachea decreased rapidly, but after 14 d it was still 0.5% of the value

immediately after inhalation. In the extrapulmonary bronchi and bifurcation sample the mass of $^{235}UO_2$ fell less rapidly until by 14 d approximately 10% of the original amount remained.

3. New autoradiographic techniques have been developed for the detection of fissionable and α-emitting particles with superimposed α-images of lung tissue sections in Lexan and CR-39 plastics. The distribution of inhaled $^{235}UO_2$ particles in the lungs of rats between 2 and 35 d after inhalation was investigated using such autoradiographs and a Quantimet Automated Image Analyser. The mass of $^{235}UO_2$ particles found in the tracheo-bronchial region relative to that in the pulmonary region was greater than that predicted by the ICRP lung model. The results suggested that between 7 and 35 d after inhalation 4% of the lung burden was associated with the airways, as opposed to 0.01% recommended by ICRP.

4. The mechanism of particle retention was studied using $^{133}BaSO_4$ particles deposited locally in the trachea. Liquid emulsion autoradiography and electron microscopy showed that particles which were not rapidly removed by muco-ciliary clearance all remained on the surface for at least 2 h, most being ingested by macrophages. By 7 d, all the particles still retained had become buried within the tracheal wall, and were all in macrophages, which were thought to have penetrated the epithelium after ingesting particles on the surface.

5. The spatial distribution of retained particles with respect to the luminal surface of the airway epithelium has also been determined. 7-17 d after the inhalation of $^{235}UO_2$, particles were found both in the lumen near the surface of the epithelial cells and within airway tissue. Between 66 and 81% of the $^{235}UO_2$ associated with the airways were within the airway tissue at a most likely depth of 8-10 μm. Similar distributions were found 1 and 7 d after local deposition of $^{133}BaSO_4$ particles in the trachea. By 7 d, 86% of the aggregates buried in the wall were within 40 μm of the epithelial basement membrane.

6. The proportion of inhaled particles retained in the airway wall is not large, but the site of retention near the basal cells of the epithelium suggests that these findings may be important in assessing the risk of lung cancer from inhaled insoluble α-emitters.

Publications resulting from this work

Patrick, G. Int. J. Radiat. Biol. 35, 571-576, 1979.
Gore,D.J. and Patrick, G. Phys. Med. Biol. 23, 730-737, 1978.
Gore,D.J., Thorne, M.C. and Watts, R.H. Phys. Med. Biol. 23, 149-153, 1978.
Gore,D.J. and Jenner, T.J. Phys. Med. Biol. 25, 1095-1104, 1980.
Gore, D.J., Ph.D. Thesis, CNAA, London, 1979.
Stirling, C. and Patrick, G. J. Path. 131, 309-320, 1980.
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Part II - CARCINOGENESIS

The results and their relevance for radiation protection

Rats inhaled $^{235}UO_2$ particles (mass median aerodynamic 2.5 to 2.8 μm and σg = 1.6 to 1.7) and were subsequently exposed to slow neutrons in a reactor thermal column. The fission of ^{235}U in the oxide particles caused localised irradiation of the lung by fission fragments and the aim of these experiments was to study whether such fission fragment 'hot particles' would induce malignant tumours of the lung.

Different amounts of $^{235}UO_2$ aerosol were inhaled by the rats giving nominal lung contents of 40 and 400 μg at 7 d post-inhalation, the ^{235}U being exposed for 2.5 min to a fluence of $\sim 10^{12}$ neutrons cm^{-2} at either ~20 h (group I) or 7 d (group II) after inhalation. There were also three control groups: Group III no $^{235}UO_2$ or neutrons, group IV $^{235}UO_2$ only and group V $\sim 10^{12}$ neutrons cm^{-2} followed ~24 h later by $^{235}UO_2$ aerosol. Each group contained 40 animals and they were kept for their lifetime.

For all the treated groups with nominal lung contents of 400 μg there was a significant increase in the number of primary malignant tumours of the lung compared with the untreated group III. Considering the animals with 400 μg $^{235}UO_2$, there was no significant difference in the yield of malignant tumours between groups I and II, which were exposed to fission fragments (~800 rads mean fission fragment dose to lung at either 20 h or 7 d following inhalation plus α-rays from $^{234}UO_2$ impurity present in the $^{235}UO_2$ retained in the lung throughout the animal's lifetime plus radiation from exposure in the reactor), and group V which received the same radiations but no fission fragment exposure. The tumours in group V were probably induced predominantly by the α-ray irradiation of the lung and not by exposure of the animals in the reactor. The mean lung dose from α-rays during the mean survival time (~600 days) of the irradiated rats was ~600 rads and at this dose the fission fragments appeared to have little additional carcinogenic

effect on the lung. The animals in the treated groups with nominal lung contents of 40 μg $^{235}UO_2$ showed no significant difference in incidence of malignant tumours compared with the untreated control group III.

The carcinogenicity of fission fragment 'hot particles' was investigated with the possibility of using them to study the 'hot particle' hypothesis for lung. The present experiments, however, have not established whether fission fragments can induce tumours in rat lung because of the high incidence of tumours caused by the α-ray irradiation.

Measurements were made of the number and mean mass of the aggregates or foci of $^{235}UO_2$ particles that formed in rat lung as a result of the phagocytic action of macrophages. The animals inhaled different amounts of $^{235}UO_2$ aerosol in a similar way to those in the carcinogenesis study so as to give lung depositions at the time of measurement that ranged from 40 to 400 μg. Thin tissue sections were cut from plastic embedded lungs of animals killed at ~20 h and 7 d following inhalation at the times when similar animals would have been exposed to neutrons in the carcinogenesis study. The aggregates of $^{235}UO_2$ particles in these sections were revealed by a method of neutron induced autoradiography. The number of particle aggregates g^{-1} lung did not increase in a linear fashion with lung content of the aerosol. Those animals with lung contents within the range 40 to 200 μg $^{235}UO_2$ showed little change in the number (~30 x 10^6) of foci or aggregates g^{-1} lung but an increase in mean mass of the aggregates occurred. The number of these foci containing $^{235}UO_2$ particles g^{-1} lung is very similar to the number of macrophages found per g of rat lung from other experiments. A significant increase in the concentration of aggregates or foci did occur, however, for lung contents between 200 and 400 μg of $^{235}UO_2$ with the concentration for some lungs increasing to ~100 x 10^6 aggregates g^{-1} lung. It is not clear whether this increase in the number of aggregates was due to an increase in the number of lung macrophages engulfing the $^{235}UO_2$ particles or if some of the particles were not being incorporated into the macrophages and were left lying free in the lung. The macrophages, however, are an important factor in controlling the mass and number of fission fragment 'hot particles' in the carcinogenesis study.

Publications resulting from this work

Batchelor, A.L. et al. Int. J. Radiat. Biol. 37, 249-266, 1980.
Jenner, T.J. and Thorne, M.C. Phys. Med. Biol. 25, 357-364, 1980.

Contractor	United Kingdom Atomic Energy Authority Atomic Energy Establishment, Winfrith, Dorset
Contract No	278 - 79 - 1 BIO UK
Head of research team	D. Ramsden
General subject of contract	Plutonium exposures in man. Direct monitoring of the lung and lung model assessments.

Project No 1

Title: The distribution of plutonium activity within the lung

Head of project and scientific staff: D Ramsden, P P Foster, K Kingman, L Somervaille

Systems have been developed in this and other laboratories for measuring the X-rays from inhaled plutonium via detectors placed externally to the chest. From such data assessments of the lung contents can be made (Ref 1). The errors and uncertainties, which arise from the low count rates, the absorption of the soft X-rays within the chest wall, the variations in the chest wall size and the interfering responses from non-plutonium body radioactivity, have been studied and quantified in the past. Such detection systems are now in routine use for plutonium-in-lung assay throughout the nuclear industry. The main source of uncertainty in present systems, is that arising from the unknown distribution of material within the lung following an accidental intake. It is estimated that extremes of distribution could produce an error in estimating lung contents of up to a factor of 3. This project is aimed at developing techniques to characterise the distribution, so that a calibration figure could be derived which would be specific to the incident, the inhaled material and the observed distribution within the lung.

The project - started in 1979 - has concentrated for the first 1½ years on the following aspects.

(a) Modification to the detectors and their associated electronics;
(b) Ultrasonic analysis of chest wall structure;
(c) Development of software for (a) and (b);
(d) Modifications to existing phantoms and procurement of alternative phantoms;
(e) The initial responses of the new configuration;
(f) Data handling.

(a) Modifications to detectors and associated electronics

These modifications, which were completed in 1980, took longer than anticipated because of the necessity to ensure that the system was always available for plutonium-in-lung measurements. However, this had the advantage that each stage was thoroughly checked and that the overall aim of maintaining a practical system for low level assessment was always paramount.

The modifications to the detectors and the shielded room have now resulted in a system with two phoswich detectors above the bed and two multiwire counters, each with two independent main counting wires, mounted beneath the 'transparent' bed. From a single session six independent responses are obtained from the subject, whilst measuring the subject in both prone and supine positions gives twelve responses which then form the input data set for further analysis. The low backgrounds have been maintained and improved throughout these changes and at present are, in the 12 to 25 keV X-ray band:-

12.5 cm diameter phoswiches	2 cpm/phoswich
Xe filled proportional counter	< 1.5 cpm/counting wire

In order to assess each detector output independently the associated analogue electronics have had to be expanded. These are now installed, fully tested and interfaced to the mini computer. The system is also interfaced to four large sodium iodide crystals used for the simultaneous measurement of body gamma activities.

(b) Ultrasonic studies of chest wall structure

The standard approach of using a single valued chest wall thickness is not adequate for this project. A detailed qualitative description of the tissue intervening between each detector and each lung volume is required. Ultrasonic chest scans on all Winfrith plutonium workers have been obtained. The tissue thicknesses are in the form of X-ray plates, are partially quantified and coded for computer storage and manipulation.

The methodology of standardising and storing the ultrasonic data has proved to be the area of greatest difficulty to date. The present approach is to store soft tissue depth, adipose tissue depths and rib depths on a standard raster, together with scaling factors obtained from external markings and chest X-rays. This approach is amenable to pictorial representation, solid angle calculations and digitalisation of lung volumes. It is not amenable for iterative analysis (Monte Carlo techniques) or for direct comparison with phantom data. A second method of storage, holding mean X-ray absorption paths in a series of two dimensional arrays is also under investigation.

(c) Software development

For this project softward development covers three areas.

(i) The real time aquisition system based on a PDP-11/34 mini-computer with CAMAC interfacing

(ii) The off-line data manipulation both on the PDP-11 and an intelligent graphics peripheral (Tekronix 4052)

(iii) Large scale data manipulation and analyses on main-frame machines (ICL 470 & 2796)

(i) The real time data aquisition programme have been extended to handle all detector inputs independently. Such programmes are fully tested and operational (Ref 2).

(ii) The off-line data manipulation (gain stabilisation/normalisation, background subtract, record and storage) routines are complete. The link with the graphics unit and digitalisation of ultrasonic work are also installed and tested, but are still under investigation with a view to further simplification and automation.

(iii) The main frame softward routines exist. This software still needs further development in order to give full statistical analysis from the planned measurement programmes.

(d) Phantoms

The Winfrith realistic chest phantom (1964) has been completely refurbished and modified, in an attempt to achieve realism from the back of the chest. Substandard projection phantoms have also been modified with the aim of giving both realism and a sufficient flexibility to match subject observations. New lungs have been obtained for these phantoms and the calibration programme for both point and extended sources is fully planned. All work on this aspect of the project was halted in mid 1980 awaiting the delivery of a new realistic chest phantom from the Lawrence Livermore Laboratories USA (1981). Preliminary cross checks between phantoms were done in 1978/79 (Ref 3).

(e) Initial measurements

During the period of the contract to date over 1000 measurements have been stored for future analysis. These measurements originate from the routine plutonium in lung assessment programme. Most of the measurements were made with 'interim configuration' and only the last group were monitored with the 'final configuration'. All the measurements, already assessed for total plutonium in lung assuming a homogeneous distribution, will be reanalysed assuming a non-homogeneous distribution in the second stage of the project.

(f) Data handling

The responses from each detector, together with their associated errors, after corrections for background and contributions from body gamma activity, are held in an array, R_i (i = 6 or 12). Such arrays exist for both the plutonium response (17 keV) and the americium response (60 keV). The distribution within the lung is expressed as j volumes and for a unique solution, i = j. (The conditions where $j < i$ are also being investigated to determine whether such an approach will decrease the overall error in assessment). Constraints as to negative values are also being introduced. The response matrix is then the form

$$R_i = \sum_j x_j E_{ij}$$

where x is the activity in lung compartment j and E_{ij} is the efficiency of detector i to compartment j. E_{ij} is of the form

$$E_{ij} = A_{ij} B_{ij} \exp (C.D)_{ij}$$

where A is the solid angle factor, B is bone (rib) losses, C is the sum of absorption in intervening tissue ($\Sigma\mu t$) and D is a modifying geometric factor for each element of C.

Formulation of the matrix and its method of solution are being investigated in the following ways:-

(i) Monte-Carlo techniques using input data from ultrasonics chest dimensions and variable lung sizes.

(ii) Thorax models summarising tissue structure and solid angles to the detector arrays from defined lung volumes.

(iii) Pertubations on the phantom responses.

The effort to date has been concentrated on approach (ii) and has included considerable effort in quantifying bone and tissue

distributions at the back of the chest.

Summary

This project aims at quantifying the major 'unknown' in plutonium in lung measurements ie the distribution of the contaminant within the lung. A successful conclusion will not in itself reduce the quoted statistical errors on a measurement but will reduce systematic errors. Knowledge of distribution and changes in distribution with time will also:-

(i) Explain many of the anomolies which occur in a routine lung monitoring programme.

(ii) Provide a basis for establishing lung clearance patterns for any individual and hence remove some of the present necessary assumptions in internal radiation dosimetry for plutonium and associated compounds.

(iii) In combination with project 2 (see below) provide a basis of observation on which to apply ICRP 26 philosophy on a routine basis to the difficult problem of the inhalation of plutonium dusts.

The project is not sufficiently advanced to draw conclusions as to the eventual outcome of the work. No fundamental problems have arisen, although the computation and data analysis are, of necessity, somewhat formidable.

References (within period of contract)

1 D Ramsden 'The accuracy of a routine plutonium in lung assessment programme'. IRPA Jerusalem 1980

2 P P Foster & J Burland 'Real-time Data handling in a Whole Body monitoring facility' - Real-time Data '79 - Berlin Oct 1979

3 Campbell G, Anderson A, Fry F, Newton D, Ramsden D 'Calibration of phoswich detectors for the assessment of plutonium in lung'. Health Physics - in press.

Title of project No 2 — Lung Model assessments

Head of project and scientific staff — D Ramsden

Published data from these and other laboratories have indicated that, following an accidental intake of plutonium oxide, predictions based on the ICRP lung model neither agree with the observed clearance patterns nor are consistent with the observed urinary excretion figures. This project is aimed at establishing revised values of the parameters within lung models in order to match such models to observed lung clearance patterns. The project is also aimed at extending such models to describe quantitatively the excretion of plutonium via urine. The overall aim is to produce flexible, self-consistent models which can then be used to calculate dose commitment to the individual, from observations of lung contents and excretion levels as functions of time since intake. It is emphasised that no changes are proposed in the fundamental concepts of the ICRP lung model, but only to the values of the constants. This work was started in 1979 and is due for completion in mid 1981.

Our observations on subjects who have accidentally inhaled high fired plutonium oxides are that, after the initial rapid clearance of the upper respiratory tract via the gut / faeces, the remaining material within the lung appears to clear with a two component exponential (typical half lives being 30 days and 300 days). After such clearance, there remains a small component with a very slow clearance rate (presumably respiratory lymph-node bound material). These observations result in a significant change in predicted dose commitment because about $\frac{2}{3}$ of the material present is excreted very much more quickly than the ICRP model suggests.

Three alternative explanations for the faster clearance were considered.

(a) The material behaves as if it were of mixed solubility (Classes W & Y in ICRP terminology)
(b) There is an intermediate component in the pulmonary clearance
(c) There is a delayed component in the transfer from the Tracheo-bronchial compartment.

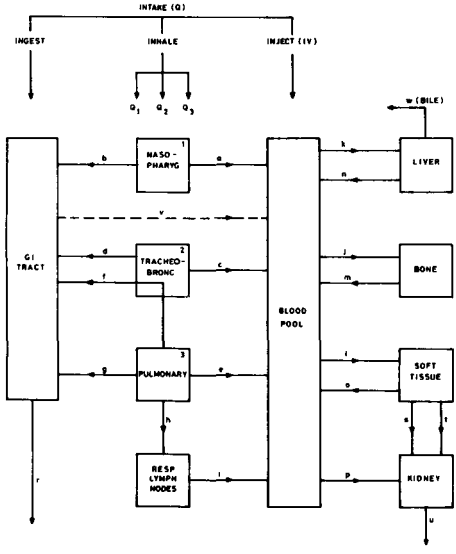
Observations on the urinary and fecal clearance from exposed individuals show that there is no soluble component (class W) and that the intermediate component is almost totally removed via the gut.

The project consists of a series of computer programmes - the UPRED series (Urinary Predictions) which are written in Fortran IV on the Winfrith IDL-470 machine. At present there are four such programmes with a fifth to be written.

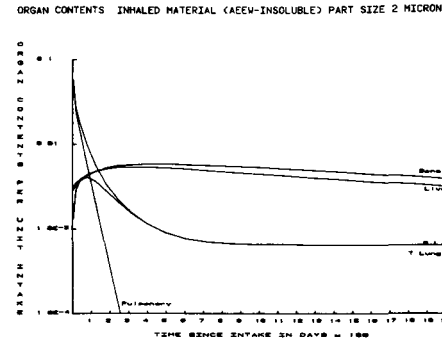
1 UPRED 1 is a lung modelling routine
2 UPRED 2 is urinary excretion routine - injected material
3 UPRED 3 is a combination of the above - urinary excretion from inhaled material
4 UPRED 4 analyses data from Winfrith's measurement programme 1960 - 1980 and compares observed and predicted urinary excretion
5 UPRED 5 analyses the results of the above routines to produce

the best model for the group and for specific individuals.

Fig 1 shows the compartmental model used for the above programmes.



1 COMPARTMENTAL MODEL



2 ORGAN CONTENTS - INHALED MATERIAL - AEEW BEHAVIOUR (2 MICRONS)

UPRED 1

The ICRP lung model can be expressed as a series of linear first order differential equations, easily soluble by standard techniques. The equations used here are almost identical to those recently summarised in ICRP publication 30. The solutions are expressed, both in tabular and graphical form, as organ contents versus time for a unit intake of defined particle size and defined breathing pattern. The input data set is taken directly from ICRP 19 and from the Task Group on Lung Dynamics.

The model, and input data set, is then extended to cover the Winfrith observed clearance pattern with the intermediate component being considered both as part of the T-B compartment and as part of the Pulmonary compartment. The preferred approach is now to consider the intermediate component to be part of the pulmonary clearance and that its transfer to blood and respiratory lymph nodes to be proportional to the length of time the material stays within the pulmonary compartment. In practice this means that for this intermediate component very little material is solubilized and clearance is via the T-B compartment and gut (see Fig 1). As an illustration of the type of data obtained from this routine Fig 2 shows some organ contents versus time for a Winfrith clearance pattern for a particle of 2 microns.

UPRED 2

The clearance of injected 'soluble' plutonium from the body and the urinary excretion pattern can be expressed as a power law (Wright-Langham). It was considered desirable to attempt to categorise such clearance by compartmental modelling (sum of exponential terms). Initial attempts to construct such models resulted in urinary excretion curves which were remarkedly similar to those reported by Rundo (IAEA

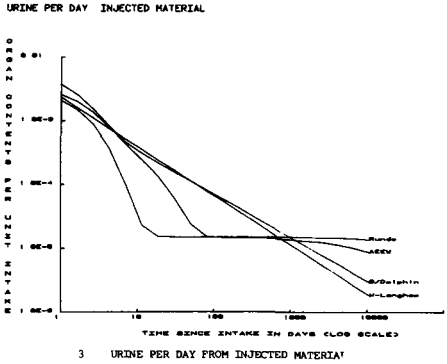
Stockholm 1971 'Assessment of Radioactive contamination in Man) on late observations from Wright-Langham's subjects. Several such models were then developed which were constrained to meet the following requirements:-

(a) The early urinary clearance to follow a $T^{-0.7}$ power law
(b) The late urinary clearance to be within the bounds of the above power law and the observations of Rundo.
(c) The apparent half-life in bone to be 100 years (ICRP)
(d) The apparent half-life in liver to be 40 years (ICRP)

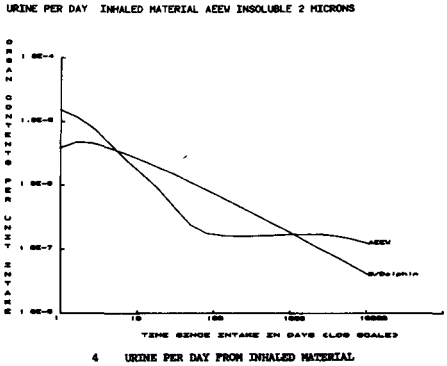
The simplest workable model (see Fig 1) has a soft tissue compartment with slow release back to blood and a two component release to a kidney compartment and hence to urine. The magnitudes of the transfer coefficients were chosen by iterative techniques. Figure 3 gives an example of urinary clearance from such models.

UPRED 3

Both the above routines are expressed as a sum of exponential terms and can be combined by standard convolution techniques to give the predictions for urinary excretion patterns for inhaled material. UPRED 3 predicts urinary excretion per day per unit intake as a function of particle size for combinations of the lung models and the urine clearance models. Typical outputs are given in Fig 4.



3 URINE PER DAY FROM INJECTED MATERIAL



4 URINE PER DAY FROM INHALED MATERIAL

UPRED 4

This programme takes observed lung contents from Winfrith personnel and predicts the urine excretion per day at any time. These predictions are then compared with observed urinary data from the same subjects. The input data set consists of measurements of lung

and urine, with associated errors,from workers in the Winfrith Experimental Fuels Laboratory over the period 1960 - 1980. Also included are dates of known or suspected intakes. The data is initially analysed to produce times of intake consistent with observations and with the known working history of the worker. The intake pattern, as a function of the lung model assumed, is then established and predicted urinary excretion patterns calculated. Recalculation based on different lung models and, where necessary, different intake regimes are then made (These calculations include the errors of the original observations).

UPRED 5

This programme, yet to be written, will select the best fit model to the individual or group of individuals. It will calculate the dose commitment, following ICRP 26 formulation. It is hoped (but has yet to be demonstrated) that the variations within the group are small in terms of the range of values of parameters within the models and the resultant effects on dose commitments.

Summary

The ICRP lung model does not match the observed lung clearance of high fired plutonium oxides in Winfrith workers. Small pertubations of the parameter within this model are sufficient to obtain a match to the observations but such small changes produce major changes in dose assessments. This compartmental model is then extended to predict urinary and faecal excretion rates.

From such models, comparison of observed and predicted data for an individual or group of individuals, enables us to select the best formulation for the dosimetry of the individual or group.

If the philosophy of ICRP 30 is to be applied, it is necessary to match the internal dosimetry component to the individual rather than a fixed single model of behaviour. This project will be completed and published in 1981.

Contractant de la Commission : COMMISSARIAT A L'ENERGIE ATOMIQUE
F.A.R.

N° du contrat : 277-79-7 BIO F
Chef du groupe de recherche : J. CHALABREYSSE

Thème général du contrat : Elucider certains points concernant la biotoxicologie de l'Uranium et préciser les normes de surveillance radiotoxicologique.

- Titre du projet =

ETUDE DE LA TOXICITE ET DU METABOLISME
DE L'URANIUM NATUREL ET ENRICHI

- Chef du projet et Collaborateurs Scientifiques =

J. CHALABREYSSE - Dr. J. CAMARASA
F. TEULON - M. ARCHIMBAUD
R. BERTRAND

Dans l'étude entreprise, trois directions de travail étroitement imbriquées et convergentes, sont poursuivies =

. Etude du polluant, des conditions réelles d'exposition et de l'environnement du travailleur.

. Etude de la Santé des travailleurs.

. Etude de l'épuration pulmonaire de l'excrétion urinaire et de l'élimination fécale de l'Uranium absorbé par le travailleur.

Le travail sur ce projet a débuté en juillet 1979.

Nous présentons ici les grandes lignes des travaux mis en place et les premiers résultats obtenus.

1/ - ETUDE DU POLLUANT ET DES CONDITIONS REELLES D'EXPOSITION DES TRAVAILLEURS

Différents types de prélèvements d'atmosphère de travail ont été pratiqués dans les ateliers où l'on traite les Concentrés Uranifères pour aboutir au Tétrafluorure d'Uranium. Nous avons étudié les aérosols uranifères sur 4 plans :

- *aspect physique*
- *composition chimique*
- *solubilité in vitro*
- *concentration en Uranium*

Les prélèvements d'air ont été effectués selon deux procédés =

. *Prélèvement statique par sédimentation dans des coupelles.*

. *Prélèvement dynamique par aspiration de l'air à travers un filtre ou à l'aide d'un Cascade Impactor qui permet une sélection granulométrique des poussières entre 20 et 0,5 µ.*

1.1) Caractéristiques physiques

Pour les poussières d'UF4, la granulométrie varie de 40 µ à moins de 1 µ. La microscopie électronique a permis de montrer que la forme des particules est hétérogène même à l'intérieur de la particule.

La densité est un paramètre extrêmement important car il intervient dans la définition du diamètre aérodynamique Dac.

Le tableau 1 indique la densité et la masse volumique mesurées.

TABLEAU N°1

	NITRATE D'URANYLE	OXYDE U_3O_8
Densité dans C cl4 g/ml	2,2 à 2,3	
Masse Volumique g/ml	0,7 à 0,8	1,7 à 1,8

1.2) Caractéristiques Chimiques

Nous avons étudié les caractéristiques chimiques des aérosols suivant

des techniques différentes : fluorimétrie - Rayons X - Spectre d'émission X - Microscopie électronique à balayage.

On mesure la teneur en Uranium par fluorimétrie.

La figure n°1 présente le spectre aux rayons X de l'oxyde U_3O_8.

La figure n°2 présente le spectre global de poussières d'uranates.

1.3) Etude de la solubilité in vitro des poussières uranifères

Après différents essais nous avons retenu la technique utilisée par "Lovelace Foundation" (1). La solution synthétique de GAMBLE lèche un sandwich de poussières de granulométrie et de caractéristiques physicochimiques connues. Le pH de la solution est de 7,3 à 37° et il est contrôlé. On mesure l'Uranium dans l'effluent.

Les résultats sont exprimés en Uranium dissous en fonction du temps et en pourcentage cumulé en poids, en fonction du temps.

Les figures n°3 et 4 indiquent les courbes obtenues pour les uranates de granulométrie hétérogène.

La figure n°5 indique la courbe obtenue pour les uranates de granulométrie comprise entre 10 et 20 µ.

Le pic observé a déjà été signalé dans plusieurs publications.
Le tableau 2 indique les facteurs de dissolution mesurés pour le nitrate d'Uranyle. On définit le facteur de dissolution de la poussière comme le produit des 2 pourcentages :

- % = $\frac{\text{quantité d'Uranium cumulé dissoute à t}}{\text{quantité d'Uranium dissoute lors de l'arrêt de l'essai}}$
- % = $\frac{\text{quantité d'Uranium dissoute lors de l'arrêt de l'essai}}{\text{quantité d'Uranium totale dans la poussière}}$

Le tableau n°2 présente les résultats d'étude de solubilité pour le nitrate d'Uranyle.

TABLEAU N°2
NITRATE D'URANYLE

GRANULOMETRIE	FACTEUR DE DISSOLUTION MESURE APRES		
	12 h	24 h	48 h
Hétérogène	0,241	0,297	0,368
20 à 10 µ	0,219	0,319	0,354
10 à 5 µ	0,199	0,296	0,409

Le facteur de dissolution mesuré après 12 h augmente avec la granulométrie Par contre après 48 h ce sont les poussières de ∅ ≠ 5 µ, atteignent donc l'alvéole pulmonaire qui seront le plus rapidement solubilisées.

(1) Kotrappa and Light Design and Performance of the Lovelace Aerosol Particle Separator The Review of Scientific Instruments V43 N°8 Lovelace Foundation for Medical Education and Research Albuquerque NEW MEXICO 87108 AUGUST 72

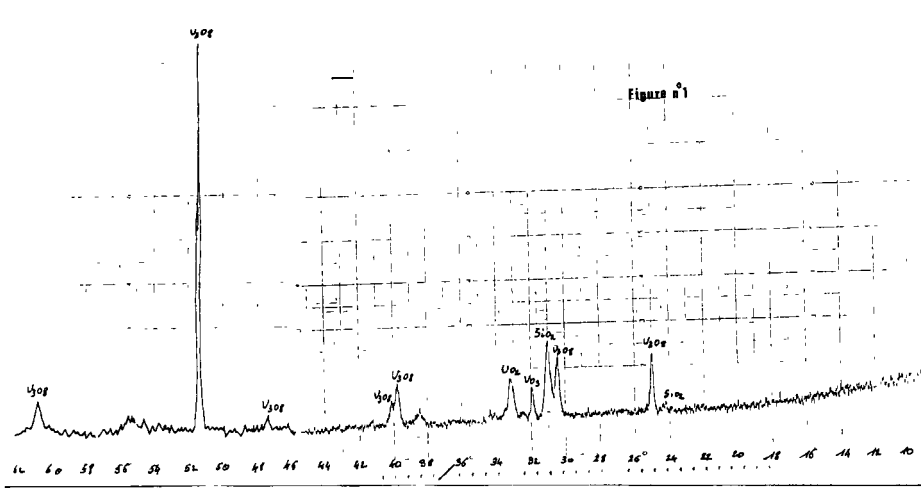


FIGURE N°1 : *Spectre aux rayons X de poussières U_3O_8 - Présence d'UO2 UO_3 SiO_2*

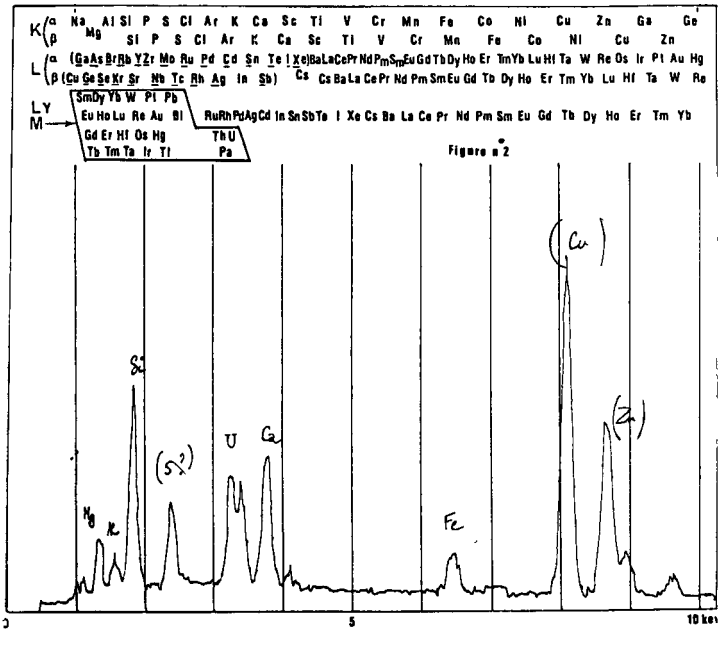


FIGURE N°2 : *Spectre d'émission aux rayons X d'uranate - Présence de Si - Ca - Fe (S - Cu - Zn proviennent de l'électrode)*

1.4) Pollution observée aux postes de travail

Nous pratiquons une surveillance systématique de l'atmosphère des postes de travail de l'entreprise d'élaboration de produits uranifères, dans laquelle nous prélevons les poussières pour l'étude de leur solubilité et nous assurons le suivi des travailleurs. Les espèces chimiques rencontées sont UO2 - U03 - U308 - UF4 - URANATES etc ... Les prélèvements d'air sont de deux types :

- *soit prélèvement d'ambiance, avec appareillage installé à poste fixe,*
- *soit prélèvement individuel, l'appareillage est alors portatif et fixé sur le vêtement du travailleur.*

Les poussières uranifères sont captées sur un filtre.
L'Uranium est dosé par fluorimétrie .
En France, la recommandation pour la valeur limite de concentration à un poste de travail est de 0,18 mg/m3. Si l'on prend en compte les résultats des teneurs en Uranium aux postes de travail que nous avons étudiés depuis Juillet 1979, on constate que 24 % des prélèvements d'air ont donné une concentration égale ou supérieure à cette valeur.

Certes des améliorations très efficaces ont été apportés aux postes de travail, en les automatisant ou en imposant le port du masque là où cela n'a pas pu encore être fait. Cette entreprise conservera néanmoins toute sa valeur de "champ d'étude" pour les intoxications par l'Uranium.

2/ - ETUDE DE L'EXCRETION DE L'URANIUM

Notre étude de l'excrétion urinaire de l'Uranium a porté sur plusieurs milliers de prélèvements en 1980 ce qui nous a apporté les informations ou confirmations suivantes :

- *l'expression des résultats par rapport à la créatinine est très intéressante et pour une étude de poste de travail et pour le suivi à long terme de chaque sujet ;*
- *l'expression par rapport à une densité de référence qui reste à déterminer est en cours d'examen ;*
- *la corrélation avec l'excrétion de calcium urinaire est en cours d'étude également ;*
- *la surveillance systématique vis à vis de l'uranium transférable par dosage sur une miction dans les 24 heures qui suivent la fin*

d'une semaine de travail, et le résultat étant exprimé par rapport à la créatinine, est très satisfaisante ;

. la période biologique de l'Uranium non transférable serait beaucoup plus courte qu'annoncée. Le taux d'Uranium urinaire reviendrait vers les valeurs de sujets non exposés (1 à 2 µg/l) en quelques semaines.

En ce qui concerne les valeurs "témoins", qui sont de très faible niveau, nous avons amélioré la sensibilité du dosage fluorimétrique par passage intermédiaire de l'échantillon sur résine liquide.

Par ailleurs, une étude sera entreprise sur ces mêmes valeurs "témoins" en fonction, peut-être de l'alimentation, et sûrement des régions géographiques.

Quant à l'excrétion fécale, le nombre de résultats est pour l'instant insuffisant. Nous sommes tout de meme amenés à nous poser 3 questions :

- un plus grand nombre de mesures confirmera-t-il les variations individuelles sur selles de 24 heures ?

- sont-elles dues seulement à des variations importantes du poids des fèces ?

- quelles sont les autres causes éventuelles ?

Nous sommes maintenant en possession de tous nos matériels pour une installation très performante d'anthropogammamétrie =

. 2 détecteurs Phoswich plus 1 détecteur Scintiflex
. La chaine électronique adéquate
. Un analyseur multicanal associé à une table traçante et un mini ordinateur.

Les travaux de réglage et d'étalonnage ont démarré.

3/ - CONCLUSION

Les travaux entrepris seulement depuis 18 mois ont déjà permis de préciser certains points essentiels = conditions d'étude "in vitro" de la solubilité des poussières, spectre de la granulométrie des poussières, validité de l'expression de l'Uranium urinaire par rapport à la créatinine, variations de l'excrétion urinaire et de l'élimination fécale des sujets témoins non exposés.

Les prochains travaux permettront d'approfondir les conditions de Surveillance des Travailleurs exposés aux composés uranifères.

PUBLICATIONS

=-=-=-=-=-=

Contribution à la Surveillance du personnel exposé à des composés d'Uranium non transférables
- J. CAMARASA - J. CHALABREYSSE - Radioprotection 1980 vol.15 n° 1 - 3 à 12

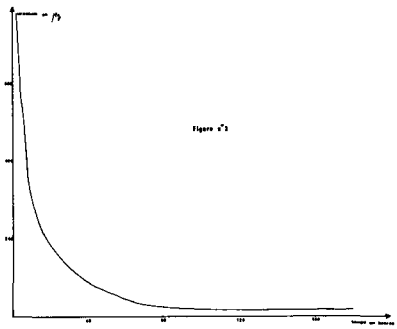


FIGURE N°3 : *Uranates Poussières hétérogènes*
Quantité d'Uranium dissoute en fonction du temps -

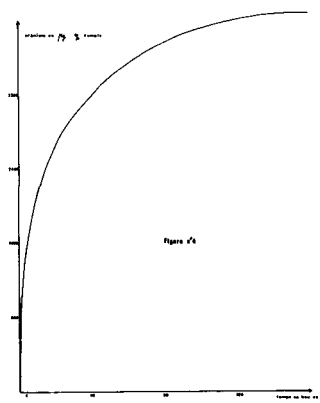


FIGURE N°4 : *Uranates Granulométrie hétérogène*
Uranium dissous cumulé en fonction du temps

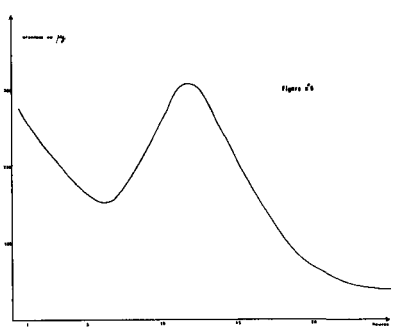


FIGURE N°5 : *Uranate granulométrie 10 µ < Ø < 20 µ*
Uranium dissous en fonction du temps.

Contractor : ENEL - Ente Nazionale Energia Elettrica
Contract number : 101-76-1 PST I
Head of Research team : Prof. Antonio Farulla
General subject : Cytogenetic researches on circulating lymphocytes of subjects professionally exposed to the hazard of ionizing radiation in a nuclear power plant station of Enel.

Title of the project :
Cytogenetic researches on circulating lymphocytes of subjects professionally exposed to the hazard of ionizing radiation in a nuclear power plant station of Enel.

Head of project and scientific collaborators :
A. Farulla - B. Dallapiccola - A. Manzoli - G. Naro

Cytogenetic analyses failed to demonstrate an increased frequency of chomosome aberrations in a group of 42 subjects occupationally exposed to ionizing radiation in a nuclear power station, as compared to a group of controls. However, the observation in radiation workers of a slight increase of acentrics as well as the sporadic observation of ring chromosomes and dicentrics, would suggest that a biological effect can be detected at cytogenetic level in subjects professionally exposed to ionizing radiations below the internationally agreed maximum permissible levels.

In any case the low frequency of such findings does not allow us to establish a quantitative relationship between chromosome aberrations and doses accumulated.

Obviously, this conclusion is valid for doses and manner of exposition superimposable to those of our cases.

From 1976 to 1980 a group of 50 radiation workers professionally exposed to ionizing radiation in a nuclear power station of Enel (Garigliano, Italy) have been monitorized by means of cytogenetic analyses in blood lymphocytes. In most subjects blood samples were obtained five times during annual medical check up.

During the first four-years follow up was shown that the mean frequency of chromatid and cromosome-type aberrations was not significantly different in the whole sample of radiation workers as compared with control groups. Further data have shown a preferential involvement of breakpoints in the interbands between Q and R bands, normal SCE rates and non-random chromosomal involvement superimposable to that found in "spontaneous" chromosome aberrations of controls.

In this paper we present the results obtained during the 1980 cytogenetic study of the same population, the correlations between chromosome aberrations rates and doses of the five years follow up in workers with accumulated doses exceeding 15 rems, and the whole conclusion of our researches.

Material and Methods.

A heparinized venous sample of 5 ml was taken during annual medical check up of 42 workers and set up in culture on the same day. Blood was cultured in Difco TC medium added with AB human serum, phytohemagglutinin and antibiotics, and incubated for 48 - 52 hours at 37° C. Chromosome preparations were performed according to Buckton and Evans (1973), using a 0,9 % Na-citrate solution for hypotonic shock. Slides were prepared by the air dried technique and stained with Giemsa. For each culture 100 cells with 45 centromeres were scored directly under microscope. Chromatid and chromosome-type aberrations were classified according to ISCN (1978).

Dose estimates (in rems) from film badges were obtained indipendently of the receipt of blood samples and after completation of chromosome analysis. The exposure involved almost exclusively gamma radiation. The accumulated doses ranged between 8 and 40 rems and the years of radiation work between 12 and 16. A parallel cytogenetic analysis was undertaken in 18 controls with superimposable age distribution.

The data derived from 25 radiation workers which had accumulated more than 15 rems (range 15-40) at the time of last cytogenetic study were analysed to establish the pattern of chromosome aberrations during the five-years cytogenetic follow up.

Results and Discussion.

The whole sample of 4.200 metaphase plates from professionally exposed individuals showed 5,13 % aberrations (including chromatid and chromosome-types), as compared to 5,05 % in 1.800 cells from controls. In both groups the rate of chromosome-type aberrations was ranging between 0 and 3 %.

Although in the absence of a significant difference in the mean frequency of aberrations in the two groups of subjects, slight differences were found in the radiation workers in respect to the controls, concerning the distribution of some chromosome-type aberrations. In the formers acentrics were detected in one of 600 metaphases, as compared to one of 900 cells in the controls. Furthermore, one of 2.100 cells in radiation workers presented either ring chromosomes or dicentrics, which never were observed in controls. Ring chromosomes were found only in individuals with accumulated doses exceeding 20 rems, and dicentrics only in workers exposed to doses over 35 rems.

In the group of professionally exposed individuals, 25 subjects had accumulated more than 15 rems at the time of last cytogenetic study. The rate of chromosome-type aberrations was increased in 15 individuals of the sample, as compared to the frequency recorded five years before. In 5 cases the rate of chromosome-type aberrations was identical, while in 5 it was decreased. Thus, about 68 % of the subjects with higher accumulated doses showed at the last cytogenetic analysis an increased proportion of aberrations as compared to that found in 1976, suggesting a dose-dependent increase in chromosome aberrations. In particular, the total number of chromosome-type aberrations increased from 0,76 % to 1,16 % in these individuals during the five years follow up ($p < 0.05$).

However, the cohomprensive analysis of 112 samples obtained from workers which at a given time of the five years study had accumulated more than 15 rems demonstrated chromatid-type aberrations only in 42 % of them. About 32 % of these workers had only one, and 18 % two chromosome-type aberrations; 8 % had three or more chromosome aberrations. Thus, a dose-dependent increase in chromosome aberrations could not be established when the mean instability rate of these subjects was compared to that of the controls through the 1976-1980 study.

Table I

CYTOGENETIC FOLLOW UP OF THE WORKERS WITH ACCUMULATED DOSES EXCEEDING 15 REMS

Subjects	Age/yr (in 1980)	Accumulated dose in 1976 (rems)	Aberrations% ct-type	cs-type	Accumulated dose in 1980 (rems)	Aberrations % ct-type	cs-type
1	43	13,5	5	-	15	4	-
2	53	13,6	6	-	15,9	5	1
3	41	12,7	9	1	19,9	7	2
4	53	18,9	4	-	20,6	4	2
5	39	14,7	6	2	20,9	4	1
6	41	17,6	3	-	21,8	2	1
7	42	20,4	3	-	21,9	1	-
8	47	18,6	4	-	22,7	8	3
9	38	19,9	6	2	21,9	6	1
10	41	19,0	2	1	23,3	3	-
11	55	21,0	5	-	23,5	4	1
12	40	21,6	6	2	24,2	6	2
13	34	17,9	2	-	24,3	4	1
14	45	25,5	5	1	27,5	4	2
15	36	22,1	4	1	28,2	1	1
16	34	21,6	5	-	28,4	6	1
17	41	22,3	4	1	28,6	5	1
18	46	26,2	8	4	29,0	8	1
19	36	22,5	4	-	29,4	4	1
20	51	25,6	4	2	30,8	4	-
21	43	27,5	8	-	32,0	4	1
22	50	28,9	5	1	35,2	4	2
23	39	30,6	5	-	35,6	7	1
24	55	29,9	7	-	36,7	8	1
25	52	33,9	1	1	40,4	9	2

Table II

SUMMARY OF CYTOGENETIC DATA DERIVED FROM 50 RADIATION WORKERS DURING THE FIVE-YEARS FOLLOW UP

Years	Radiation Workers		Controls		Other findings in radiation workers
	No. of examined cells	Percent of aberrations	No. of examined cells	Percent of aberrations	
1976	5000	5.30	2500	4.90	Preferential involvement of some bands in breaks; C-banded cells suggest preferential clustering of breakpoints in C-positive bands
1977	4400	5.14	2500	4.92	Sequential Q and R staining of chromosomes demonstrates that most breaks take place in the interbands between Q and R bands
1978	2000	5.11	2000	4.99	The rate of SCE among radiation workers is not significantly different from that of controls
1979	6534	4.55	9668	4.13	The pattern of distribution of breakpoints in chromosome closely accords in radiation workers with that of normal population
1980	4200	5.13	1800	5.05	

Synthesis of the results obtained during the contractual period (1976-1980).

The chromosome instability rate, as evaluated from the analysis of 22.134 cells of 50 radiation workers with accumulated doses ranging from 8 to 40 rems appeared grossly superimposable to that found in 18.488 metaphases of controls.

Studies of banded chromosomes have been carried out to investigate some biological features of chromosome breakage in the workers. In particular, the distribution of breakpoints in respect to the chromosomes, chromosome bands and their location within a given band was examined and compared to that of control population.

Although preliminary data showed preferential involvement of some chromosome bands in workers (3p14, 2q22, 4q32, 5p14, 13q21), later on it was found that the pattern of distribution of breaks along the chromosomes accords with that derived from general population, with a significant excess of breaks in chromosomes 7 and 14 ($p < 0.001$) and a deficit of breaks in chromosomes 6 and 8. The analysis of G banded cells gave evidence for an excess of breakpoints in the Q/G positive bands. However, to rule out the possible biases of such an analysis, sequential Q and R staining was performed on the same cells, which showed that breaks take place almost exclusively in the interbands between Q and R bands; the same pattern is now considered the general rule of most constitutional and acquired chromosomal breaks. Furthermore, the number of exchanges per chromosome per person, as evaluated by the analysis of SCE distribution in 765 cells from workers, failed to evidentiate significant difference in respect to that found in 500 mitoses of controls.

The only difference at the cytogenetic level between radiation workers and controls was the qualitative pattern of some chromosome-type aberrations, like acentrics, ring chromosomes and dicentrics, which were slightly overrepresented in the formers. This finding suggests that a biological effect may be detected by a cytogenetic approach in individuals chronically exposed to low levels of ionizing radiation.

Obviously, the low frequency of chromosome-type aberrations demonstrated through the entire period of our study cannot be correlated with accumulated doses in radiation workers with rate and manner of exposure superimposable to those of our sample.

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Contractor : Centre Anticancéreux Régional, Fondation Bergonié, F-33076 Bordeaux Cedex.

Contract N° : 242.76.7. BIO F

Head of research team : Dr J.F. Duplan

General subject of Contract : Role of leukemogenic viruses in radiation-induced leukemogenesis.

Results of Project N° 1

Head of Project and scientific staff : J.F. Duplan, B. Guillemain Th. Astier, E. Legrand and R. Daculsi

Title of Project : Analysis of the viral components of a leukemogenic viral complex recovered from radiation-induced leukemia.

The project on the role of leukemogenic viruses in radiation-induced leukemogenesis of the C57BL/6 mouse was undertaken in 1976 with the purpose of analyzing the possible role of endogenous retroviruses in radio-leukemogenesis.

In a first step in was shown that most of the cell lines which were established in vitro from radiation-induced leukemic cells did not retain their ability to produce viruses for more than a few passages. This loss of viral expression was generally related to a gradual evolution of the morphology of the cell layer suggesting a positive correlation between the presence of round cells -not fibroblastic- and the viral production. It was finally possible to derived from an in vivo passaged virus (RadLV-Rs) a cloned cell line (13-3C) characterized by a high level of viral expression. The similarity of the effects produced either by RadLV-Rs or 13-3C virus led to consider that 13-3C was already producing a viral complex containing the leukemogenic component of RadLV-Rs. This was further confirmed by molecular hybridization which demonstrated that 13-3C and RadLV-Rs had similar sequences although they were different from Rauscher or Frend leukemogenic viruses. It was further proven that both 13-3C and RadLV/Rs contained at least two main components one B-tropic and the other X-tropic. The endogenous N-tropic virus of the C57BL was seldom recovered from the leukemic tissus and it never induced leukemia

when injected into C57BL mice. On the opposite the B tropic component did show the same leukemogenic potency as this of the passages RadLV-Rs.

Finally three components B, N and X were isolated in vitro using their host range properties. Two B-types isolates were recovered. The first one infected and grew efficiently on fibroblastic cells, but was not leukemogenic. The second was isolated on TAC cells. These cells originated from C57BL thymus, they possessed neither the theta antigen nor TdT activity characterising T lymphocytes. Both infected TAC cells and the B virus which they produce were highly leukemogenic. Several clones derived from the TAC B-isolate were obtained and specific techniques were developed to improve the titration of these B viruses either by XC test or by transformation of S+L- cells. The different clones

Association between host-range, polykaryocytosis and leukemogenic activity

Origin of viruses	Host range[1]	XCEP[2]	XC[3]	Leukemia[4] induction
Rad LV-Rs	N	-	+	-
	B_1	-	+	L^l
	B_2	+	+	L^r
	X	-	-	-
3T3[5]	-	-	-	-
3T3-1223[6]	B	-	+	L^l
TAC[7]	-	-	-	-
TAC-1691[8]	B	+	+	L^r
TAC-98[9]	B	-	+	L^l
SCl[10]	-	-	-	-
SCl-1223[11]	B	-	+	L^l
SCl-1691[12]	B	+	+	NT[13]

[1] Capacity to infect and replicate in $Fvl^{b/b}$ or $Fvl^{n/n}$ cell lines
[2] Polykaryocytes produced in less than 24 h.
[3] Polykaryocytes produced after 72 h.
[4] - : non leukemogenic ; L^l : Late leukemia (100% in 500 d.) ; L^r : early leukemia (100% in 50 d.)
[5] Mouse fibroblasts
[6] 3T3 cells infected with one B virus from Rad LV-Rs
[7] C57BL thymic cell line
[8] TAC infected with 2 B viruses from Rad LV-Rs
[9] TAC infected with one B virus from Rad LV-Rs
[10] Cell line from wild mouse
[11] SCl infected with the B virus produced by 3T3-1223
[12] SCl infected with the B virus produced by TAC-1691
[13] Not yet tested.

of B-tropic viruses were throughly analyzed and it became obvious that they were actual viral recombinants. Four of them are now produced in large amounts, their structural proteins and glycoproteins have been isolated and specific antisera have been raised against each of them.

In parallel with these studies on the viral complex involved in the leukemic process, experiments were carried out in vivo to characterize the transformed cells and to investigate at the cellular level some of the radiation effects which might be relevant to the leukemogenic process. It was found that the cloned B-tropic virus induced mainly null leukemias (non-T, non-B). In addition it was demonstrated that the latency of the leukemias induced by the B-tropic viruses depended on the spontaneous expression of the X-tropic virus. This xenotropic component was necessary for the completion of the recombinational event B-X which leads to the formation of a leukemogenic recombinant. Finally a recent series of experiments suggested that after irradiation mice express the X component much earlier than control animals.

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B. GUILLEMAIN, T. ASTIER, R. MAMOUN and J.F. DUPLAN.- In vitro Production and Titration Assays of B-Tropic Retroviruses Isolated from C57BL Mouse Tumors Induced by Radiation Leukemia Virus (RadLV-Rs) : Effect of Dexamethasone. Intervirology, 1980, 13, 65-73.

Contractant de la Commission : CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE - MOL

N° du contrat : 241-77-1 BIO B
Chef des groupes de recherche : J. R. MAISIN

Thème général du contrat : COMPARATIVE STUDIES ON VARIOUS POPULATIONS OF RADIATION-INDUCED LEUKEMIA VIRUSES

Titre du projet n° 1 : Molecular Biology of a RadLV-derived rat-propagated leukemogenic virus

Collaborateurs scientifiques : M. JANOWSKI,
J. MERREGAERT

INTRODUCTION

Two distinct leukemogenic virus isolates were obtained from radiation induced tumors of the C57BL mouse. RadLV/Ka (propagated in cell cultures as RadLV/VL_3), isolated by Kaplan and coworkers, is thymotropic and induces thymic lymphomas. RadLV-Rs (propagated in cell cultures = the so-called 3c virus), obtained by Duplan and coworkers, is splenotropic and elicits mixed cell malignant lymphomas (reticulum cell sarcomas). These experimental systems could be considered as good tools to investigate, not only some possible relationships between some properties of viral particles and the tissue specificities for the malignant transformation that they induce, but also the possible viral etiology of radiation induced leukemogenesis in mice.

On the other hand, a somewhat similar comparative system was obtained following attempts to obtain abundant virus particle production by propagating RadLV-Rs in rats. A virus complex was obtained (BL/F) which could elicit, according to the experimental conditions, either a generalized malignant lymphoma (EL) killing the rats in less than 6 weeks, or a slowly developping leukemia (SL) in the form of either a thymic lymphoma (TL) or a reticulum cell sarcoma (RS). Experiments were performed to determine whether this multiple pathogenic effect could be attributed to the occurrence of distinct viral entities in BL/F or to a dose-response effect of one single virus according to the experimental conditions of inoculation. Since the first alternative proved to be correct, we derived clonal isolates from BL/F when feasible, to perform a comparative study of some of their respective properties.

BIOLOGICAL PROPERTIES OF BL/F

EL, TL and RS could to some extent be induced independently from each other, as if a distinct viral entity were responsible for each type of disease (Ref. 10). Indeed, EL spleen extracts or serum induced 90-100% EL when injected intraperitoneally. Dilution or intrathymic inoculation resulted in a progressive disappearance of EL in favor of SL. Undiluted SL extracts induced mainly TL and, occasionally, RS. The virus progeny from BL/F - infected rat embryo fibroblasts preferentially induced RS, while that of infected mouse Balb/3T3 cells exclusively induced TL.

The pathogenic titers of EL serum were, by intraperitoneal injection, 10^3 for EL and 10^6 for SL. Those for SL serum were 10^0 for EL and 10^5 for SL, in the same conditions of inoculation. These findings led us to produce clonal isolates of both TL and RS viruses, while cloning EL virus appeared to be unfeasible because of its low proportion. Not surprizingly the gross properties of EL-associated virions did not differ from those of SL-associated virus particles, as described below.

The in vitro measured infectious titers of EL-inducing serum or extracts were 10^4 to 10^6 times higher than those of SL preparates, although particle numbers were roughly identical (Ref. 10) and displayed the same reverse transcriptase activity. The only sound explanation for this phenomenon is that the EL-inducing virus might enhance the infectivity of the SL-inducing entities, perhaps through some kind of helper-defective virus interaction. The evolutions of virus infectivity during the development of EL or SL strikingly differed from each other (Ref. 10,12), emphasizing again the existence of distinct leukemogenic agents.

MOLECULAR GENOME HOMOLOGIES BETWEEN BL/F AND 3C VIRUS

Molecular hybridization experiments were performed to evaluate the genome homologies between the bulk component of BL/F and that of the 3c virus, using the endogenous Gross MuLV and the exogenous Rauscher MuLV as reference systems. In vitro synthesized ^{3}H-DNA's, complementary to the genome of

each investigated virus, were hybridized to each of the 70s RNA genomes investigated (Ref. 2,7). BL/F and 3c displayed no more than 50% homology, probably because of the fact that 3c consists of a complex mixture of several endogenous C57BL viruses in roughly equivalent proportions. Both the virus preparates were more similar to Gross MuLV than to Rauscher MuLV.

SEQUENCE HOMOLOGIES BETWEEN BL/F, 3C, AND MOUSE AND RAT DNA'S

Hybridizing the BL/F and the 3c ^{3}H-DNA probes with normal or leukemic mouse spleen DNA's resulted in 90-100% homology (Ref. 3,7). The rat-grown virions did not appear to contain detectable rat cellular DNA sequences, while multiple copies of their nucleotide sequences were detected in covalent linkage with infected rat spleen DNA (Ref. 7,8). These results, together with those from the hybridization kinetics of the viral probes with mouse DNA (Ref. 7), strongly support the endogenous mouse origin of the investigated virions.

SEROLOGICAL SPECIFICITIES OF CLONED BL/F ISOLATES

Clonal isolates were obtained from BL/F and characterized serologically as described in Table I and Ref.10, by using them as competitors in double antibody radioimmune competition assays. The reactions were those specific for the p12, p30 and gp70 proteins of known prototype viruses (Balb virus 2, NIH MuLV and AKR MuLV). The bulk of the EL-inducing particles displayed, as expected, the same properties as those of its major constituent (TL-inducing virus, cloned on either Balb/3T3 or SC-1 cells). They were those of a B-tropic virus, derived by recombination between the 3 prototypes investigated. The RS-inducing virus, selected by propagation on rat embryo fibroblasts and cloned on SC-1, did not differ from the former to a measurable extent, except at the level of its p30 protein, responsible for NB-tropicity. The TL- and RS- inducing viruses differed however strikingly from each other where their RNase T1 oligonucleotide fingerprints were concerned. Their gp 70 envelope constituents did not belong to any of the known prototype viruses, including the thymotropic RadLV/VL$_3$.

TABLE I - Recombinant nature of cloned BL/F viruses

Viral isolate	Serologic specificity				Tropicity (in cell cultures)
	p12	p30	gp70	αK36::::	
BL/F (EL extract)	Balb 2	not done	unique::	-	B
→ clone on Balb/3T3	not done	NIH	not done	-	B
→ clone on SC-1	not done	NIH	not done	-	B
grown on REF	Balb 2	NIH	unique	±	NB
↓ clone on SC-1	not done	NIH	unique	±	NB
BL/F (SL extract)	Balb 2	not done	unique	not done	

:: negative in assays for Rauscher MuLV, Moloney MuLV, Balb:2, NIH MuLV, AKR MuLV and RadLV/VL_3

:::: assay indicator for tropicity, complete competition indicates N tropicity, partial competition NB tropicity and no competition B tropicity.

CONCLUSION

Propagation of RadLV-Rs in rats resulted in the abundant production of a virus complex, containing viral entities responsible for a generalized malignant lymphoma (EL), a thymic lymphoma (TL) and a reticulum cell sarcoma (RS), respectively.

The properties of the major constituent (that responsible for TL) were those of an endogenous C57BL mouse virus, generated in a genetic recombinational process between various endogenous prototype viruses. We still ignore whether this event occurred during the evolution of the C57BL mouse strain (in which case irradiation would merely activate the expression of the corresponding, genetically transmitted provirus), as a consequence of irradiation or of development of lymphoid cells into a malignant state (which could be investigated by restriction enzyme analysis of the mouse genome), or as a consequence of the interaction between viral entities in the RadLV-Rs complex during propagation in rats (which also could be checked by restriction enzyme analysis).

The viral entity responsible for the induction of reticulum cell sarcomas (RS) could also be selected and cloned. It did not appreciably differ from the former where the investigated serologic properties were concerned, except at the level of the p30 antigenic determinant associated with viral tropicity. Oligonucleotide fingerprints revealed, however, specific characteristics.

One major feature of the clonal isolates from BL/F is the unique type-specificity of the envelope gp70 constituents, which differ from that of RadLV/VL_3. The specificity of virus-induced leukemogenesis might reside at that level, as suggested previously in the literature.

The origin of the various leukemogenic virus recombinants found in BL/F will be investigated by performing restriction enzyme analyses of C57BL mouse DNA, isolated from normal tissues as well as from radiogenic and virus induced tumors. The results of these experiments hopefully will shed some light on the still discussed viral etiology of radiation induced leukemia.

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Contractors:	Laboratory of Tumor Biology University of Copenhagen
Contract No.:	256-77-7 BIO DK
Head of Research Team:	Dr. Keld Danø
General Subject of Contract:	Pilot Study of the Role of a Plasminogen Activating Enzyme in Strontium-90 Induced Tumors in Mice

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Synthesis and release of plasminogen activators seem to be implicated in a variety of physiological and pathological processes involving extracellular proteolysis. These include mammary gland involution, trophoblast invasion, follicular rupture during ovulation, macrophage and granulocyte migration, and expression of the malignant phenotype. A likely function of plasminogen activators in malignant neoplasia is participation in the generation of localized tissue destruction during invasive growth. The aim of the present study is to examine the possible role of plasminogen activators in the growth of radiation induced tumors, by enzymatic and immunohistochemical examination of the occurrence of plasminogen activator in strontium-90 induced tumors in mice, and by an attempt to prevent the occurrence of tumors in mice after strontium-90 treatment, by administration of rabbit antibodies against plasminogen activators. For these studies pure enzyme is necessary for the preparation of specific antibodies in rabbits. We have isolated a clone of cultured mouse 3T3 cells infected with mouse sarcoma virus. These cells produce 40 fold more plasminogen activator than uncloned cells. The enzyme has been purified and characterized, antibodies with a high degree of specificity have been raised in rabbits, strontium-90 induction of tumors in mice has been started, and techniques for detection of the enzyme in mouse cells by the immune peroxidase procedure have been developed.

Purification and characterization of a 48,000 Dalton plasminogen activator from mouse cells transformed by an oncogenic virus.

On the basis of cellular morphology, a subline of mouse sarcoma virus infected 3T3 cells was selected which released a 48,000 Dalton plasminogen activator at an approximately 40-fold higher rate than those of the parent line, and which continued to do so for several months when the cells were maintained in serum-free culture medium. Culture medium containing 0.6 mg/l of the enzyme was used to purify the enzyme 130-fold with a yield of 32% by affinity chromatography followed by anion exchange chromatography and gel filtration. Crucial for the yield was the use of a non-ionic detergent and of inhibitors of proteolysis to prevent adsorption and degradation, respectively. The purified enzyme was homogenous as evaluated by SDS-polyacrylamide gel electrophoresis and had an isoelectric point of pH 9.2. The purified enzyme showed characteristics of a trypsin-like serine protease (labeling with ^{3}H-diisopropylfluorophosphate which was prevented by p-nitrophenyl-p'-guanidino benzoate) and converted the single chain of human plasminogen into two chains of plasmin with electrophoretic mobilities identical to those of the chains formed by non-purified enzyme and by human urokinase. In the absence of inhibitors, solutions of purified enzyme were stable for 24 hours at 4^{o} at pH 3-9.

Recent studies have shown that under serum-free conditions the enzyme is released from the cells as an inactive proenzyme consisting of one polypeptide chain. Plasmin was found to convert the proenzyme to an active protease consisting of two polypeptide chains with molecular weights of 19,000 and 29,000 Dalton, the latter containing the active site.

Production and characterization of rabbit antibodies against 48,000 Dalton mouse plasminogen activator.

Precipitating antisera were raised in rabbits against the electrophoretically pure 48,000 Dalton mouse plasminogen activator. The IgG fraction of the antisera inhibited 48,000 Dalton mouse plasminogen activators from a variety of sources (neoplastic and non-neoplastic), a 29,000 Dalton

plasminogen activator from mouse urine and a 48,000 Dalton plasminogen activator from rat urine. No inhibition was observed of a 75,000 Dalton plasminogen activator extracted from mouse lung, of mouse plasmin or of plasminogen activators from human urine and from oncogenic virus-transformed chicken cells. The IgG antibodies are stronger and more specific inhibitors of the 48,000 Dalton mouse plasminogen activator than any previously tested compounds.

Studies on strontium-90 induced osteosarcomas.

Malignant osteosarcomas have been induced in CBA/C57Bl mice by treatment with strontium-90. Extracts from all osteosarcomas which have been induced until now (eighteen) have contained 48,000 Dalton plasminogen activator as detected by enzymatic methods.

Methodological studies have been performed with cultured murine virus-transformed fibroblasts on the use of the peroxidase-antiperoxidase technique with antibodies against plasminogen activator to detect the enzyme histologically. By this method the enzyme can be detected in the above mentioned highly producing line of mouse sarcoma virus infected 3T3 cells, while the method is not sensitive enough to detect the enzyme in the strontium-90 induced osteosarcomas. Preliminary studies have shown that this is due to the fact that the 48,000 Dalton plasminogen activator intracellularly is present as a proenzyme (see above), while the antibodies are directed against the active enzyme and only show a low degree of cross-reaction with the proenzyme. Antibodies against the pro-plasminogen activator are now being raised with the aim of obtaining a more sensitive immunohistochemical method for detection of the proenzyme.

Systemic inhibition of 48,000 Dalton plasminogen activator in mice by administration of antibodies.

Studies on the toxicity in mice of the antibodies to the plasminogen activator have been performed. These show that mice without adverse effect can be treated intraperitoneally with the antibodies in an affinity purified form daily for five consecutive days in doses that give a complete systemic inhibition of the enzyme throughout a period of 144 hours.

This finding shows that it is possible to use the antibodies to test whether the enzyme plays a role either during carcinogenesis or for invasive growth of tumors. Such studies will require large amounts of affinity purified antibodies which presently are being produced.

Conclusions.

This pilot study has shown that strontium-90 induced osteosarcomas as all hitherto studied malignant murine tumors contain a 48,000 Dalton plasminogen activating enzyme. The enzyme has been purified and characterized. Antibodies that inhibit the enzyme strongly and with a high degree of specificity have been produced and found non-toxic in doses that inhibit the enzyme systemically in mice for 6 days. It is concluded that the pilot study warrants continued investigations of the role of this proteolytic enzyme for the induction of tumors by irradiation and for the invasive growth of these tumors. Indispensable new knowledge and tools for such continued studies have been obtained.

Publications.

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Danø, K., Oronsky, A. and Gjedde, S.: Proteases from Cultured Malignant Cells. In: Regulatory Proteolytic Enzymes and their Inhibitors. S. Magnusson, M. Ottesen, B. Foltmann, K. Danø and H. Neurath (Eds.) Pergamon Press. Oxford 1978, 113-125.

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Danø, K., Møller, V., Ossowski, L. and Nielsen, L.S.: Purification and Characterization of a Plasminogen Activator from Mouse Cells Transformed by an Oncogenic Virus. Biochim. Biophys. Acta 1980, 613:542-555.

Danø, K., Nielsen, L.S., Møller, V. and Engelhart, M.: Inhibition of a Plasminogen Activator from Oncogenic Virus-Transformed Mouse Cells by Rabbit Antibodies against the Enzyme. Biochim. Biophys. Acta 1980, 630:146-151.

Contractor:	University of Copenhagen Institute of Medical Microbiology
Contract No.:	251-77-1 BIO DK
Head of research team:	Peter Ebbesen
General subject of contract:	Influence of Ageing Alterations on Tumor Development after Beta-irradiation

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Background:

Ageing influences the tumor development after tissue exposure to carcinogen by providing the time span required by latent periods, by providing time for repeated exposure and time to intrinsic changes in tissue susceptibility to carcinogenic stimuli. The present contract aimed at studying the intrinsic changes in susceptibility to tumor induction by irradiation that might result from ageing. Previous studies had shown that senescent mouse skin was more prone to tumor development after exposure to chemical carcinogen than younger mouse skin (Ebbesen, Science 183:217-218, 1974; J. Nat. Cancer Inst. 58:1057-1060, 1977). One factor contributing to this age-dependent change in susceptibility could be change in skin content of mitotic inhibitors. We found senescent skin, even when grafted to young mice, to have a reduced content of extractable epidermal mitotic inhibitor (Olsson and Ebbesen, Exp. Geront. 12:59-62, 1977). Further study of this mitotic inhibitor, therefore, was included in the project.

The initial objectives were 1) to determine if ageing of mouse skin caused a change in susceptibility to the carcinogenic effect of beta-irradiation and 2) to determine if any change in susceptibility was correlated to change in susceptibility to tissue specific mitotic inhibitor. As the project proceeded faster than anticipated we added the third objective, 3) to determine if ageing of mouse skin caused a

change in susceptibility to the tumorigenic effect of ultraviolet irradiation.

The first objective to be achieved was the correlation between skin ageing and in vivo skin susceptibility to tissue specific mitotic inhibitor. It was found (Ebbesen, Olsson and Due, Exp. Geront. 13:365-367, 1978) that ageing abrogated the susceptibility to the mitotic inhibitor, presumably an example of loss of local regulatory capacity since it occurred in senescent skin although grafted onto a young recipient.

This view was supported by our finding that cloned malignant cells in vitro contain less mitotic inhibitor and were less susceptible to inhibitor than their normal parental cell and revertant cells (Ebbesen and Olsson, Brit. J. Cancer 38:732-736, 1978).

Subsequently, we found that mouse skin beta-irradiated 3 months previously held more of the mitotic inhibitory compound than un-irradiated skin. We believe disturbances in such local regulatory processes may be part of the long-term effects of irradiation (Due and Ebbesen, in press).

The second objective was studies on BALB/c mice. Skin from untreated old (14 months) and young (2 months) donors were grafted to young recipients and the mice then left untouched for 8 months. Thereafter, the animals received 4200 rads of beta-irradiation on the grafts. Similarly, old and young non-grafted animals were irradiated. Skin tumors developed with a higher incidence on senescent than middle-aged skin irrespective of whether grafted or not (Ebbesen, Int. J. Radiat. Biol. 37:563-567, 1980). The fact that grafting to a young mouse does not alter the age-dependent change in susceptibility demonstrates that it is a local, autonomous process.

Ultraviolet light was used as carcinogen in the last experiments, the objective of which was to see if mouse skin also got more susceptible to this carcinogen when getting old. This is a carcinogen that mice normally are not exposed to due to their fur and nocturnal activity. The experimental set-up was as with the study of beta-irradiation. We found once more an age-dependent increase in tumor incidence when

comparing middle-aged with senescent skin (Ebbesen and Kripke, submitted).

The objectives thus have been achieved. Their relevance for radiation protection is this. Ageing may be associated with increase in susceptibility to various carcinogens including irradiation. This may have various explanations but can not solely be a result of previous exposure as our mouse skin until time of experiment was shielded from UV light. Other unknown carcinogens may be at work but very likely intrinsic autonomous, local changes take place in the skin irrespective of external changes. The fact that transfer of old skin to young recipients had no influence on susceptibility to carcinogen also demonstrates that factors as immune surveillance, hormone and viral status outside the skin are of no importance.

This all suggest that if human beings mimic the mice radioprotection for senior workers must be rigid. Their remaining expected lifespan is lower than for younger adults but their susceptibility to the carcinogenic effect of irradiation may be higher than for younger adults.

References:

Ebbesen, P. , Olsson, L. & Due, C.: Altered epidermal mitotic rate response to extract of young skin in senescent graft skin and associated younger recipient mouse skin. Exp. Geront. 13:365-367, 1978.

Ebbesen, P. & Olsson, L.: Specificity of the DNA-synthesis inhibitory effect of extracts from cloned normal, revertant and sarcoma virus transformed 3T3 cells. Brit. J. Cancer 38: 732-736, 1978.

Ebbesen, P.: Increased susceptibility to beta-irradiation of senescent mouse skin irrespective of grafting to young recipients. Int. J. Radiol. 37:563-567, 1980.

Due, C. & Ebbesen, P.: Change in activity of fibroblast mitotic inhibitor from mouse embryo cells aging in vitro and undergoing spontaneous transformation. Exp. Geront. 15:315-320, 1980.

Ebbesen, P. & Kripke, M.: Age, sex and site influencing ultraviolet light skin tumorigenesis in mice. Submitted for publication.

Due, C. & Ebbesen, P.: Mouse skin beta-irradiated 3 months earlier shows increased content of 30-50 000 MW inhibitor and decreased susceptibility to exogenous inhibitor. Anti-cancer Res. (in press).

Contractor : Radiobiological Institute TNO, Rijswijk
The Netherlands

Contract no. : 253-77-1 BIO N

Head of Project : J.J. Broerse

General subject of contract : Biological effectiveness of neutron radiation for carcinogenesis.

Investigations on radiation carcinogenesis are essential for assessing the risks of ionizing radiation during occupational and accidental exposure. These types of studies are also important to allow risk-benefit analyses of diagnostic procedures involving small doses of ionizing radiation. With few exceptions, the human data available for X- and gamma-irradiation pertain to relatively large doses. In order to extrapolate such data to the range of much smaller doses, as required for risk estimates of X-ray diagnostic procedures and industrial radiation exposure, the shapes of the dose-effect curves for tumour induction and the influence of external factors must be evaluated.

Results obtained from our previous studies on mammary tumour induction in several strains of female rats following single dose irradiations with X rays and monoenergetic fast neutrons have been published (Broerse et al., 1978; van Bekkum et al., 1979). In the present supplemental program the effects of single dose irradiation are compared with those observed after irradiation in 5 fractions, spaced by one month intervals. The studies have been performed with X rays and 0.5 MeV neutrons, for which the highest RBE values are to be expected. The following groups each consisting of 40 female WAG/Rij rats have been introduced into the program: a) controls, b) 200 rad X rays, single dose, c) 5 x 80 rad X rays at one-month interval, d) 20 rad 0.5 MeV neutrons, single dose and e) 5 x 6 rad 0.5 MeV neutrons at one-month intervals.

The irradiations were started when the rats were 8 weeks of age. All animals were examined once weekly for mammary tumour development and general physical condition. They were followed until natural death or when they became moribund. Some rats were sacrificed when their mammary tumours became so large as to interfere with their normal daily activities. All rats that were not severely autolyzed were necropsied. Partial necropsies were done and the following tissues were routinely sampled: all mammary tumours or two mammary glands in the absence of tumours, liver, lung, spleen, mesenteric and peripheral lymph nodes, and pituitary gland. The uterus, cervix and

vagina were studied histologically only when lesions were found grossly.

The results have been analysed following life table techniques. The possibility of animals surviving past time t_k without known tumour is recursively calculated from:

$$P(t_k) = P(t_k\text{-}1) \cdot \frac{n_k - \delta_k}{n_k} .$$

For this calculation distinct observation times t_k are considered, $P(t_0) = 1$, n_k is the number of animals at risk during the interval (t_{k-1}, t_k), being the number of animals alive without diagnosed tumor at t_{k-1} and δ_k is the number of animals showing a first tumor in the interval (t_{k-1}, t_k). The cumulative tumor prevalence can be calculated as $1\text{-}P(t_k)$. A few examples of the life table curves are presented in figures 1 and 2.

The cumulative prevalence values for the occurrence of all types of mammary tumours at 22 months of age in the irradiated groups are greater than the control value. However, the values for the various irradiated groups do not differ significantly from each other. The cumulative prevalence is similar for rats irradiated with single doses of X rays and 0.5 MeV neutrons, implying that the RBE at this level of prevalence approaches 10. The cumulative prevalence values for the fractionated irradiations are also similar which indicates the occurrence of repair processes in the X-irradiations as well as in the neutron irradiations. The cumulative prevalence values at 22 months of age are in good agreement with that found in a previous study involving single dose irradiations in this rat strain.

Table 1 lists the frequencies of some nonneoplastic and neoplastic lesions found in a selected number of other tissues examined in these rats. Notable is the absence of significant differences in pituitary tumour development in the five groups. The only lesion found with increased frequency in irradiated rats was the endometrial polyp; the increased frequency of neoplasms of the reproductive tract in neutron-irradiated rats was not statistically different from that of control rats. Of interest is the relatively small number of lymphoreticular tumours found in all groups and the fact that there was no apparent increase in their number after irradiation with X rays and 0.5 MeV neutrons. If this finding is corroborated in other rat experiments presently in progress, it may suggest that alternative models for studying radiation-induced lymphoreticular and myeloid malignancies should be sought in species other than the rat or in a rat strain other than that used in these studies.

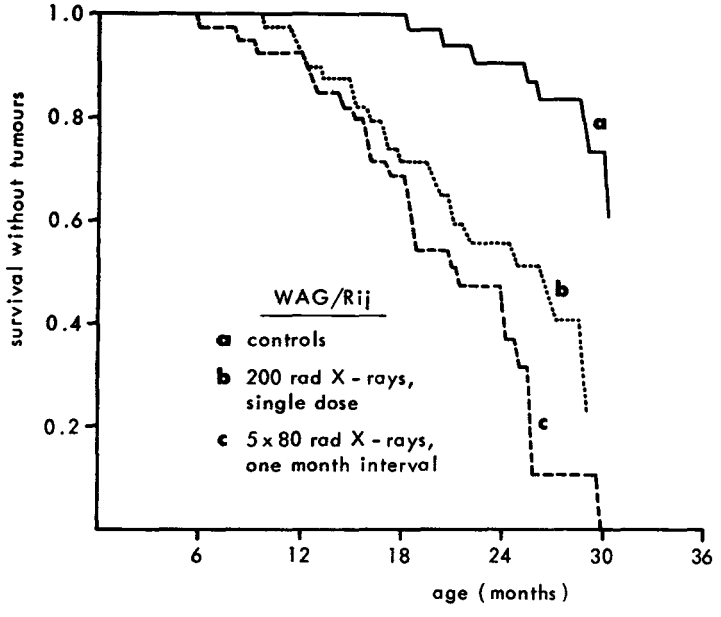


Figure 1.

Probability of rats surviving without mammary tumours after single and fractionated irradiations with X-rays as a function of time.

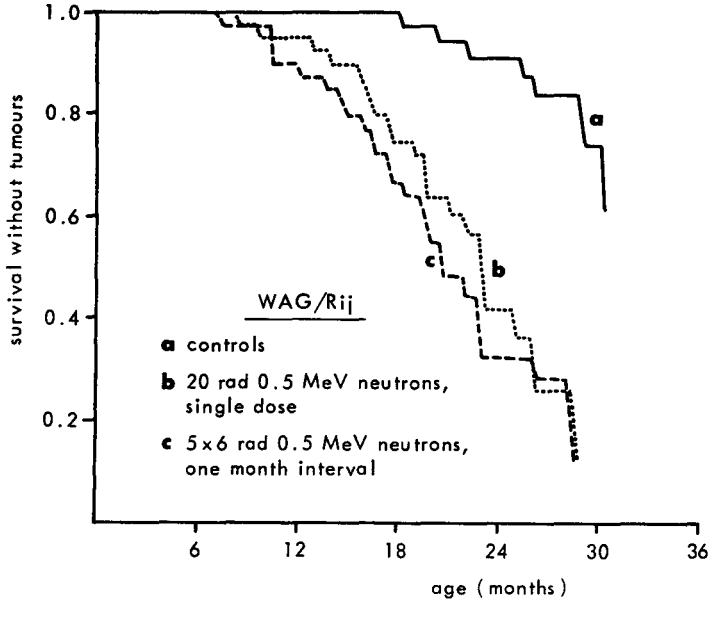


Figure 2.

Probability of rats surviving without mammary tumours after single and fractionated irradiations with 0.5 MeV neutrons as a function of time.

LIST OF PUBLICATIONS Contract no. 253-77-1 BIO N

Bekkum, D.W. van, Broerse, J.J., van Zwieten, M.J., Hollander, C.F., Blankenstein, M.A. (1979). Radiation induced mammary cancer in the rat. p. 743. In: Proc. Sixth International Congress of Radiation Research, Tokyo.

Broerse, J.J., Knaan, S., van Bekkum, D.W., Hollander, C.F., Nooteboom, A.L. and van Zwieten, M.J. (1978). Mammary carcinogenesis in rats after X- and neutron irradiation and hormone administration. p. 13. In: Proc. Symp. on Late Biological Effects of Ionizing Radiation, International Atomic Energy Agency, Vienna.

TABLE

Neoplastic lesions in selected tissues of WAG/Rij following single and fractionated dose irradiation.

Lesion	controls	1x200 rad X rays	5x80 rad X rays	1x20 rad 0.5 MeV neutrons	5 x 6 rad 0.5 MeV neutrons
Pituitary gland adenoma	26/38 (68)*	18/32 (56)	18/29 (62)	19/30 (63)	11/30 (37)
Uterus/cervix/ vagina	3/43 (7)	1/38 (3)	0/38 (0)	4/40 (10)	6/39 (15)
Lymphoreticular neoplasms	2/43 (5)	3/39 (8)	5/38 (13)	0/40 (0)	1/38 (3)
Lung neoplasms	0/43 (0)	0/39 (0)	0/38 (0)	1/40 (3)	2/39 (5)
Liver: neoplastic nodules	12/43 (28)	12/39 (31)	8/36 (22)	4/39 (10)	6/39 (15)

* No. with lesion/no. examined histologically (per cent).

Vertragspartner der Kommission: G S F - Neuherberg.

Nr. des Vertrages: 207-76-7 BIO D.

Leiter der Forschungsgruppe: H. Kriegel.

Allgemeines Thema des Vertrages: Decorporation of radionuclides

Leiter des Projektes: W.E. Kollmer, GSF.

Wissenschaftliche Mitarbeiter: W.H. Müller, CEC, decorporation,
G. Jackl, GSF, biochemistry,
W. Schmahl, GSF, pathology.

Titel des Projektes: Decorporation with cryptating agents and related compounds.

The following points of our working program of 1980 have been persecuted respectively realized during 1980 :

- In our efforts, concerning search for new decorporating effective agents for the removal of radionuclides, we were successful in two directions, 1) the testing of new substances from external institutions,2) the built-up of an installation, which permits to do the difficult synthesis of cryptating agents by high-dilution technique in our own laboratory. A first pilot-synthesis was already successful. Therefore we are now able to synthetize the C^{14}-labeled cryptand (222), necessary to establish the planned (222)-pharmacokinetics, which we could not realize up to now because of the extremely high price, demanded by external suppliers.
- We extended our decorporation experiments with cryptand (222) on Ba^{133}. This work was paralleled by Ba^{133} metabolism analysis in the rat. Both studies permitted to extrapolate the obtained treatment scheme from rat to man.
- Toxicological as well as pharmacokinetical aspects of (222) were further explored.
- The new substance (222.41), which we developed in our laboratory is most promising to become a potent and selective decontaminant for toxic and radiotoxic cations in water and intestine. Though all chemical properties are not yet studied, the qualities already known are of high interest. Therefore an application for a legal protection of this compound and its applica-

bility is under way.

RESULTS 1980

A) Search for new decorporating agents.

The very remedy for removing radioactive cadmium respectively for cases of a poisoning with inactive cadmium has not yet been found. For this reason TACY-13-TA and DTPA were compared with respect to Cd^{109}. It is as effective as DTPA, as far as the total-body retention of cadmium is concerned, however the critical organ in Cd-poisoning, the kidney is 50 % less burdened in the case of a TACY-13-TA treatment. This promising result (see annual Report 1977) is of interest for further study.

B) Chemical studies.

1) A new gravimetrical method, in order to determine the decorporant cryptand (222), was developed. The method is useful for aquous systems, extendable to other cryptands and to biological fluids.

2) IN VITRO stability studies were undertaken in order to know more about the possible IN VIVO resistance of the recently developed decontaminant (222.41) with good results. (222.41), for wich legal protection is applied, is able to remove Sr, Ba and Ra and potentially Cd, Pb, Tl, Hg and Ag from water as well as from the intestine. (see Report 1976-1980).

C) Decorporation studies with cryptand (222).

A Ba^{133}- metabolism analysis combined with decorporation studies using cryptand (222) in rats gave the following results:

1) There is no difference between an intraperitoneal or an intraveneous incorporation of the radionuclide, if one analyses the blood-retention of Ba^{133} as a function of the time. A kinetic study with a C^{14}-labeled (222) will give answer on the question if the i.p.- or the i.v.-way of decorporation is preferential for a nuclide involved.

2) Ba^{133} decorporation with cryptand (222) is limited by the uptake of the nuclide into bone. Therefore the Ba^{133} retention in the skeleton of rats was also analysed as a function of the time.

3) Graph 1 shows the Ba^{133} excretion, normed on the controls as 100 % retention during a (222)-treatment with different (222)-doses and after different time lags of treatment start. In using the known blood retention as time function of Ba-burdened man, an extrapolation from the obtained treatment scheme in rats, to man was possible. (see Report 1976 - 1980).

4) Isotrodym, a neodym containing drug, which removes Ca from its protein binding site, did not enhance the Sr^{85} excretion provoked by (222) in mice.

D) Toxicology of cryptand (222).

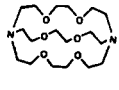
1) The marketable (222), (Kryptofix 222), may contain four byproducts, 1) (222)-diamid, 2) (222)-diboran, 3) (222)-monoxide and 4) (222)-dioxide. By means of mass spectrometry the byproducts 2), 3) and 4) were found. The dioxide as well as the diboran (though boron is only present up to 0.01 %) were synthetized and tested in mice. They did not show a significant increase of acute toxicity compared with (222) itself. The diamido forms of the cryptands are much less toxic than the free diamines as it could be shown, after the synthesis of (223)-diamid, in mice.

Conclusion : the mentioned by-products in the marketable (222) do not seem to influence remarkably the i.p. toxicity threshold of (222).

2) It was not possible to shift the toxicity threshold of (222) by Atosil in mice. (Histamine hypothesis).

E) Pharmacokinetics of (222).

Serum electrophoresis on acrylamid gel (pH 7.4) of a (222) contaminated human serum revealed, that ± 100 umole of (222) per liter blood is bound mainly to the albumine fraction migrating in kathodic direction, whilst all (222) higher than 100 umole per liter walks into the anodic direction and is not bound to any protein.



(222)

RESULTS 1976-1980.

An important subject of the Radiation Protection Program during the period 1976 - 1980 was the Decorporation of Incorporated Alpha-, Beta- and Gamma-Emitters in Mammals, because removal of radionuclides means radioprotection by reduction of internal radiation doses.

Though we tried to apply new perceptions, techniques and substances useful for decorporation on the removal of all hazardous radionuclides respectively their model nuclides, our efforts were mainly focussed on the radioactive alkaline earthes $Sr^{90/89/85}$, $Ba^{140/133}$ and $Ra^{226/224}$.

Pollution of mammals with radionuclides leads into a dynamic process of resorption and transportation, which can be stopped by the therapist at three different successive time sections or levels of penetration into the body :

I) The level of contamination, which concerns the pollution of external or internal body surfaces.

II) The level of incorporation, which concerns the pollution of body fluids and organs, with a rapid metabolism of the nuclide.

III) The level of deposition, which concerns finally the pollution of the target organ or critical organ with an inert metabolism of the nuclide.

Our efforts were directed on all three kinds of corresponding techniques of removal, which are :

1) Decontamination,
2) Decorporation and
3) Remobilization.

These techniques demand differing methods as well as differing therapeutics for one and the same radionuclide.

We should like to summarize only some of the main results of our activities in these three directions :

The Decontamination Studies summited in the discovery of substance (222.41), a derivative of cryptand (222), first synthetized in our laboratory. This unsoluble product removes radio-strontium and radio-barium very rapidly to about 99 % from aqueous solutions. On radium it is less effective. In preliminary animal experiments it showed a high efficacy in removing Sr^{85} from the intestinal tract if contamination and treatment was applied orally (100 - EQ X 100 = 90,3 %; dose ± 90 umole/kg). (222.41) is most promising to become a potent and selective decontaminant of other toxic and radiotoxic elements, for example Hg, Cd, Pb and Tl .
Though all chemical properties are not yet studied, the already known qualities are of high interest, for instance for water-chemists and in decontamination. Therefore we now apply for legal protection of this compound and its applicability.

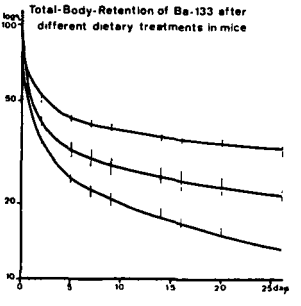
Decorporation Studies were mainly and systematically focussed on the deepening of our knowledge concerning cryptand (222), up to now the only agent well potent to remove the radionuclides $Sr^{90/89/85}$, $Ba^{140/133}$ and $Ra^{226/224}$ from mammals as far as the nuclides are not yet deposited in the skeleton. This we could point out in the past (1,2,3,4,5). In rats the decorporation effects of cryptand (222) towards the mentioned radionuclides were studied as function of all parameters (Dose, time, stability constant(6)) involved.
These results permitted the elucidation of the theoretical background of decorporation with cryptating agents in general. It resulted in the formulation of the Schubert-Catsch-Heller-Relationship, an equation, which permits to predict decorporation effects of cryptands in mammals (11). This equation made it possible to determine the therapeutic ranges (7) of cryptand (222) towards radio-Sr, radio-Ba and radium in rats.
By means of all obtained data we were able to get a first but still ventureal picture of a treatment scheme for the decorporation of radio-strontium, radio-barium and radium with cryptand (222) in man , which permits the following tentative conclusions:
a) Early treatment start is absolutely necessary !
b) In case of radio-barium incorporations, high decorporation effects are possible already with low (222)-doses, which may not even show pharmacological side-effects of (222).

c) Elevated (222)-doses will render a good removal of radiostrontium; the toxicity threshold of (222) eventually permits its application to humans in cases of Sr^{90} incorporations.
d) In cases of radium incorporations the toxicity threshold of (222) is in conflict with its applicability.

As an outstanding result it was further shown in lactating rats, that (222) prevents the uptake of Sr^{85} by the suckling pups (10)

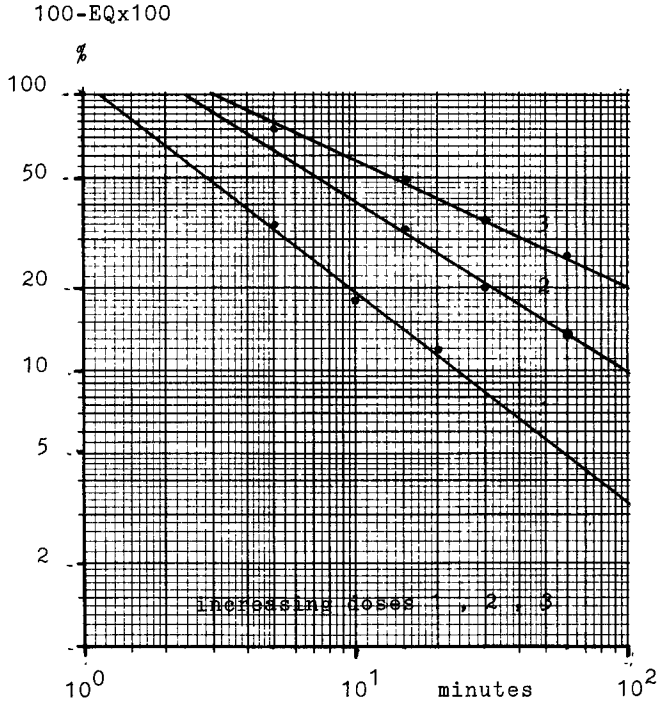
From Remobilization Studies only one result is presented here, (annual Report 1978) obtained in mice.
Target organ of radioactive alkaline earthes is the skeleton. Using Ba^{133} as a model nuclide, a surprising augmentation of barium excretion was shown with the combination of two known dietary techniques for which the same mobilizing mechanism was postulated (Graph).

This may be a tentative model for an application in case of a delayed treatment start after intoxications with the radionuclides Sr, Ba and Ra,already deposited in the skeleton.



All these experiments in animals reveal already, that each of the three techniques of removal separately applyed, do not completely exhaust the corresponding level of pollution. Therefore it seems necessary to apply a successive combination of all three techniques, in cases of severe contaminations with radioactive alkaline earthes.
One may mention, that part of our research has still an initiating and/or preliminary character. Though we reached already numerous insights into the difficult task of radio-alkaline earth decorporation, we are still far from the end of efforts necessary.
As closing remarks one can say :

Not all aimes of our program 1976 - 1980 have been reached, for example the (222)-C^{14} kinetics is still missing (see annual Report 1980).
Concerning the question of relevancy of our work for radiation protection, the brief summary of our main results obtained during the period of contract, may give an illustrating answer.



Ba^{133}-EXCRETION NORMED ON CONTROLS = 100 % RETENTION AFTER A DELAYED TREATMENT START WITH (222) IN RATS

Datas obtained from total-body retentions on the 7-th day after Ba-incorporation & (222)-treatment

G R A P H 1

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Contractor of the Commission : BELGIAN NUCLEAR CENTRE, SCK/CEN, B-2400 MOL.

N° of contract : 233-77-1 BIO B
Head of the research team :
O. VANDERBORGHT, Ph.D.

General subject of contract :
MOBILIZATION AND SPECIFIC DECORPORATION OF HEAVY ALKALINE EARTH METALS.

Title of project n° 1 : Physiological mechanisms of mobilization and excretion of the heavy alkaline-earth metals.

Head of projects and scientific staff :
O. Vanderborght, G. Schoeters,
S. Van Puymbroeck, L. Kestens.

Research was continued to decorporate heavy alkaline earths such as strontium and radium. Strontium has still to be considered as a potential hazardous radiocontaminant and ^{226}Ra remains a standard element to be referred to in radiotoxic experiments with internal emitters. The potential hazards of the radioactive heavy alkaline earth metals seems us to be relatively more important than could be inferred from the amount of decorporation research spent on this subject, as compared with decorporation studies on other radioactive substances.

In our laboratory we demonstrated that strontium could be mobilized by a chronic treatment with Na-alginate (a combination of guluronic and mannuronic acid extracted from brown seaweeds). Even several weeks after ^{85}Sr injection in mice, when the ^{85}Sr was already fixed in the skeleton, its daily excretion and its concentration in blood could be doubled by treating the animals with Na-alginate mixed with the food. Calcium metabolism of the treated animals was not affected, the urinary ^{85}Sr concentration was somewhat lowered.

In the past five years the studies concerned several aspects of decorporation.

1. Further optimalization of decorporation

In this respect a combined treatment of alginate administered both in intraperitoneal injections and in the diet with a starch containing dough, increased up to 5 times the concentration of ^{85}Sr in the blood of mice with a 9-week old radiostrontium contamination. This combined treatment overcame the decrease of urinary ^{85}Sr excretion, formerly obtained with alginate diets. The availability of the mobilised ^{85}Sr for renal excretion, for fecal excretion and for equilibration between blood and organs was different depending on the mobilizing treatment (injection or diet). The radiostrontium content of liver, kidney and spleen was also increased four to six times by the combined treatment. The experimental data allowed to estimate that the biological half life of strontium is about halved by the use of alginate in the diet combined with alginate injections. Alginate in the diet alone will fasten the decorporation of ^{85}Sr from the skeleton with about 40%

2. Comparison between ^{85}Sr, ^{226}Ra and ^{210}Pb mobilization by Na-alginate after an old contamination

A three months lasting alginate containing diet could reduce the ^{226}Ra content of a mouse femur without causing decalcification. The same amount of ^{226}Ra was taken out of the femur by the alginate treatment, independently of the total radium content of this bone.

Oral alginate treatment enhanced stronly the ^{226}Ra content in blood and faeces of mice i.p. injected with radium, and unlike with strontium it does not lower the urinary excretion of radium.

Na-alginate showed to be a non-toxic well tolerated food-additive and it was also used and proved successfully to decorporate ^{210}Pb. Radioactive lead(^{210}Pb)was intraperitoneally injected in C57Black mice.

Three latency times were allowed for ^{210}Pb to distribute in the organisms, then 5 mobilising treatments were started. The results indicate that alginate added to the diet can increase to more than 80% the ^{210}Pb in blood; it can also increase the urinary and faecal excretion. This increase is most pronounced in mice with the older ^{210}Pb-contamination. It is independent of the age of the mice at ^{210}Pb-contamination.

3. The study of physiological mechanisms by which Na-alginate acts to decorporate strontium and radium from the skeleton

The increased ^{85}Sr excretion from an old body-burden, caused by a Na-alginate diet could only be explained by the presence of Na-alginate in the intestinal lumen and not by the uptake of alginate derivatives in the blood. This is supported by three arguments: (1) no alginate derivatives could be detected in the blood; (2) no change was observable in the ultrafiltrability of strontium in the serum; (3) the increase of the ^{85}Sr concentration in the blood was paired with a decrease of the concentration of stable strontium in the blood. The third observation supported the hypothesis that the increased ^{85}Sr concentration in the blood during an alginate diet was due to a shift in the stable Sr-equilibria between the intestinal lumen, the blood and the skeleton to the left.

The doubled ^{226}Ra level in blood after Na-alginate treatment occured also together with a decrease of stable strontium in the blood as was measured by plasma emission spectrometry.

PROJECT N° 2: LONG-TERM THERAPEUTIC TREATMENT OF RADIUM CONTAMINATED ANIMALS

Head of project and scientific staff: G. Schoeters, S. Van Puymbroeck, O. Vanderborght

Whether a therapeutic decorporative treatment really improves the health and survival chance of radiocontaminated animals has to be evaluated by its eventual prevention or reduction of late radiation effects. It seems that this basic statement is often overlooked in short-term decorporation attempts.

The increase of ^{85}Sr and ^{226}Ra levels in blood after Na-alginate treatment made us worry about the eventual therapeutic value of a long-term Na-alginate treatment. Research on the eventual value for the health of the treated animals was also based on the following idea. The radiosensitive tissues (bone marrow cells, bone cells and their progenitors) localized at the interface of bone and bone marrow are hit by α-particles (soft tissue range: $\pm$ 30 µm for ^{226}Ra) emitted from a very small part of the total amount of radium which is rather homogeneously distributed in the bone volume. Reduction of late radiotoxic effects may then be proportional to the small amount of radium eliminated from the bone surface, thus from the target region. If decorporation by treatment removes only a small amount of the radioisotope but which is localized near the sensitive tissues, then the therapeutic value may exceed the beneficial effects which may be predicted from the simple relationship between "total amount of radioisotopes present in the whole bones" and " the amount of radium which is decorporated".

In this respect two study lines were developed in the past five years, connected 1)with bone tumor inductions and 2) with protection of bone marrow stem cells.

1. Reduces a long-term treatment with Na-alginate bone tumor induction in ^{226}Ra contaminated mice?
   The appearance of bone tumors is known to be a sensitive parameter of radiation harm to bone cells, even at low doses. In 1978 three series of C57Bl were injected with increasing radium doses causing (as known from literature) an increasing amount of osteogenic tumours. This experiment consisted of 850 mice, including 250 non-contaminated control mice. Half of the contaminated mice and half of the non-contaminated mice were treated with Na-alginate. Presently all mice are dead. Autopsies and röntgenphotos of the carcasses were taken. Histological and statistical analyses are in elaboration.

   Meanwhile the tolerance of animals on Na-alginate as a food-additive (6% of their diet consisted of Na-alginate) was followed in C57Bl mice and BALB/c mice. The animals were kept on a daily diet with Na-alginate for 30 weeks. No changes were detected in body-weight,in the calcium content of the femur, blood and spleen. The number of peripheral red blood cells remained unaltered, but the number of peripheral white blood cells was significantly decreased in mice treated with Na-alginate.

2. Are haemopoietic marrow cells less affected in mice treated with Na-alginate after ^{226}Ra contamination?
   Bone tumors can only be detected at the end of the life of the animals. The study of haemopoietic cells has the advantage that at any time after contamination in any marrow site, e.g. the number and concentration of the stem cells can be investigated. The haemopoietic cells are susceptible to irradiation, and damage results in haematological disorders which can be measured quantitavely.

   Experimental assay systems for in vivo and in vitro culture of resp. the pluripotent stem cells (CFU-s) and granulocyte committed stem cells (CFU)c were used .

Three-month-old male BALB/c mice were injected intraperitoneally with $^{226}RaCl_2$ at dose levels of 4.5, 6.9, 9.0 and 13.5 $\mu Ci^{226}Ra/kg$ body wt. At the two highest doses, the number of multipotential bone marrow stem cells was severely depressed 8 weeks after the injection. By 30 weeks no depression was observed compared to controls. The number of peripheral red blood cells was never altered, while the number of white blood cells was slightly depressed after 8 weeks of contamination. Mice fed every other week with standard pellets and on alternate weeks with a diet containing 6% Na-alginate (first given 12 days after the injection of $^{226}RaCl_2$) showed a significant reduction of stem-cell depression 8 and 12 weeks after contamination in three of the six treatment groups with manifest radiation effects on the stem cells.

These data support the hypothesis that Na-alginate stimulates removal of ^{226}Ra mainly from the endosteal bone surfaces, reducing the local ^{226}Ra dose which accounts for damage to marrow stem cells at the endosteal surfaces within the range of α-rays.

Experiments were started to obtain a detailed picture of spatial and temporal changes in the haemopoietic cell population of radioactive bones. This information can contribute to the knowledge of the local effects of α-irradiation and its modification by decorporative treatment. Marrow sites from various bone regions with a different Ca and ^{226}Ra metabolism were investigated. Dissimilarities between these marrow cavities were already detected in non-irradiated mice. This suggests that differences in radiosensitivity between various marrow regions are very well possible. The concentration of colony-forming cells (CFUs) is about 40% less in sternal marrow than in the marrow of lumbar vertebrae and femora. Marrow of trabecular bones in lumbar vertebrae contains fewer mitotically active CFUs than marrow of trabecular bones in the femoral distal epiphysis and metaphysis, or the peripheral marrow near the cortical bone in the femoral diaphysis.

In conclusion, the decorporation of ^{85}Sr and ^{226}Ra in animals with an old radiocontamination could be made succesful by a non-toxic and readily accepted treatment. We remember that alginate treatment was already tested in humans and omnivorous animals (swine) shortly after or together with the contamination.

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Contractor: Medical Research Council, Cyclotron Unit, Hammersmith Hospital, London. W12 OHS.

Contract No: 151-76-7 B10 UK.

Head of Research Teams: T. JONES.

General Subject of Contract:

Exposure to Radiation from Medical Diagnostic Procedures.

General Description of work carried out from 1976 - 1980.

A principle means for advancing diagnostic procedures in Medicine lies in formulating methods to observe, non-invasively, the patho-physiological disturbance that occur within tissues of the body. The use of penetrating ionizing radiation offers the opportunity to examine the internal structures of the body. The transmission of photons from an X-ray source provides information on regional tissue density; which is known to change in pathology. The emission of photons from radio-isotopes, introduced into the body to trace specific physiological pathways, provides measurements of local tissue physiology. In turn tissue disturbances are reflected directly in these measurements of function. The information which these transmission and emission procedures provide is unique. However, their diagnostic use needs to be qualified against the resulting radiation doses to the body tissues. The aim of the contract was to assess the value of these methods for examining disease of the brain, heart and lungs. Initially an all encompassing analysis of both emission and transmission was planned. However our institute is playing a leading role in the development and use of radionuclide procedures in Medicine. This arises both from the Cyclotron facilities that exist for manufacturing radionuclides with the hospital, and the research orientated environment for developing methodology relating to these isotopes. Hence the contract has concentrated on the centre's expertise in the radionuclide application, since cross reference to radiographic methods can be obtained using reports from many centres equipped with the more common X-ray facilities. The contract deals with methods for studying regional cerebral, cardiac and pulmonary function.

In order to minimise the radiation dose to the patient, short-lived radionuclides have been used. These fall into two categories : single photon emitters generated from long-lived radioactive parents and cyclotron, positron emitting isotopes. Two categories of instrumentation have been used for measuring the in-vivo distribution of tracer. In

the initial period of the contract, gamma ray cameras and probes provided two dimensional measurements of the tracer distribution. However in the interest of obtaining the third dimension and hence higher detection accuracy, two emission computerised axial tomographs were installed in the institute at the latter stages of the contract period. One was tailored specifically for the single photon emitters; a rotating gamma camera and the other for coincidence detection of the paired annihilation photons arising from positron capture. The impact of this state of the art instrumentation is presented in this report. Future indications of improvements with respect to reducing patient dose by increasing detector sensitivity are discussed.
The work covered in the contract can be considered to be the growing edge of the diagnostic uses of radionuclides. Effort has concentrated on the development of these new procedures and on assessing the diagnostic information they have to offer. In addition, consideration has been given to the associated radiation body burdens. There emerges an ongoing picture of new techniques being applied to clinical diagnostic problems. Already in Europe other centres are planning similar studies. Hence it is envisaged that a number of these applications may become more widely practised and hence represent additional or alternative sources of radiation dose to the clinical population. The information resulting from the contract prepares the community for this eventuality. The emphasis of the contract has been on the radionuclide studies. Hence a comparison of the diagnostic information arising from alternative sources of patient radiation exposure, such as in radiology, has not been comprehensively covered in the projects. A more complete consideration of this may provide the subject of future contracted research.

Project No. 1.

Head of Project: Professor R.E. Steiner.

Scientific Staff: Mr. T. Jones,
Mr. J.D. Heather,
Mr. K.R. Butler.

Title of Project:

Assessment of current radiation load from medical exposure.

The radio dosimetric considerations in the contract have rested principly with the determination of patient radiation absorbed doses, resulting from diagnostic radionuclide studies. Radiological X-ray procedures are well established in most medical centres, and numerous reports exist on the patient doses arising from these. However the radionuclide studies developed and used in this contract are new, and by and large, are confined to hospitals equipped with cyclotron facilities needed to provide short-lived isotopes. The diagnostic procedures described in projects 2, 3 and 5 were developmental and hence concern was focussed on the patient doses associated with these tests.

In the first phase of the contract, dosimetry calculations were performed for the two dimension gamma camera studies of the heart, lung and brain. These mainly involved the short-lived single photon emitters : $^{81}Kr^{m}$, and $^{99}Tc^{m}$, which have half-lives of 13 seconds and 6 hours respectively. However many brain studies with the camera were performed using the positron emitter oxygen-15 which has a 2.1 minute half life. In these camera measurements, the degree of contrast available is low due to the two dimensional presentation of the three dimension distributions of the tracer. Hence, beyond a relatively low statistical level of data collection, little additional information can be obtained and therefore, patient doses may be kept to a minimum. The actual patient absorbed doses have been reported in the publications produced on the isotopic methods and their applications, and hence are to be found in context with the diagnostic data.

The era of emission tomography introduced the need for higher radiation loads. These result from the additional counting statistics necessary to effect accurate computed reconstructions. In the case of long-lived tracers such as Tc-99m (6hrs $T_{\frac{1}{2}}$), the additional statistical information required for tomography can be obtained without additional patient dose - the scanning times are simply extended. However, for the short-lived isotope studies, which involve tracers being administered continuously during the time of the scans, longer scanning times mean higher patient

doses. Principle emphasis has been concerned with a positron emission tomograph (PET), which was used for brain, heart and lung studies. (Projects 2,3 and 5). In addition to the increased patient doses, there emerged an important additional dosimetric feature. This arises from the fact that the PET machine provides reconstructed tomograms of the absolute tissue concentration of the tracer. This is due to the ability to correct, for the effect of tissue attenuation of the emitted annhilation photons. Thus for the first time, tissue dose calculations could be carried out using actual measured concentrations of radioactivity rather than estimated values; based on the amount of tracers administered, and approximations to the subsequent body distributions.

The positron emitters used have short half-lives (2-20 mins.). Hence their use for clinical studies is attractive in that they are effectively only present in the patient during the length of the scan. However the tissue capture of the positrons, which have energies up to 2 MeV, constitutes a large deposition of tissue absorbed doses per disintegration. In the lung, brain and cardiac studies discussed in projects 2,3 and 5, in which positron emitting isotopes were used, the tissue concentrations were measured at the activity levels which were necessary to effect positron emission tomography. The resulting dose calculations were in turn submitted to the Administration of Radioactive Substance Advisory Committee (ARSAC); United Kingdom. In all cases permission was obtained for the clinical studies to proceed. It was found that the oxygen-15 brain, and the carbon-11 labelled blood pool studies constituted the highest radiation dose procedures. In these cases the critical organs were the lungs (2 Rem) and liver (0.9 Rem) respectively. The use of Gallium-68 (68 mins. $T_{\frac{1}{2}}$), labelled microspheres for lung perfusion studies was not pursued because of the high radiation doses. In all the other procedures the doses were acceptably low, and have been published in context with the technical and clinical reports of the described methods. The patient absorbed doses are of course highly dependant on the sensitivity of the Positron Emission Tomograph used. For these studies, the most accurate whole body, PET Scanner currently available, was involved. However the machine records only one transaxial slice at a time, and hence data is recovered over a small emission solid angle. There clearly is a case for improving the sensitivity for detection. Following this experience of patient absorbed doses versus demand for tomography, the position indicates a need for improved detectors. This has already

been realized in the latest generation of positron emission tomographs designed specifically for brain studies. An order of magnitude increase of sensitivity has been reported by the manufacturer of these machines. The experience reported from this institute provides information which should be considered by centres who are embarking on the installation of such facilities. If due attention is paid, then the patient radiation loads can now be predicted, given the clinical questions being asked, and the instrument which is to be used.

Project No. 2.

Head of Project	:	Dr. J.M.B. Hughes/Dr. F. Fazio.
Scientific Staff	:	Dr. J.P. Lavender, Dr. M. Silverman, Professor P.E. Steiner, Dr. P. Wollmer, Dr. C.G. MacArthur, Mr. T. Jones, Dr. G. Giofetta, Mr. J.C. Clark, Dr. M. Miniati, Mr. C.G. Rhodes.

Title of Project:

Exposure to Radiation in pulmonary diagnostic procedures.

Within the terms of the contract consideration has been given to the development, use and value of radioisotopic procedures to diagnose lung disease. In particular, techniques for studying regional pulmonary blood flow, ventilation and water space have been covered,since these are central to the lung's function. The sensitivity of these methods for identifying focal lung disease have, where appropriate, been compared to the normal chest X-ray radiograph. The information presented can be compared to the cost; namely the patient radiation absorbed doses that are associated with the measurements. The radioisotopes used were of two categories : generator produced, single photon emitters (Krypton-81m, Technecium-99m and Indium-113m) and positron emitting isotopes produced on line by the Cyclotron (oxygen-15, nitrogen-13, and carbon-11). Most of the pulmonary measurements involved the use of conventional gamma ray cameras which provided two dimensional images of tracer. However near the end of the contract, two emission computerised axial tomographs were used : one specifically for positron emitting and one for the single photon category of isotope. Prior to the initiation of the project, this institute had pioneered a new method for producing high quality images of regional pulmonary ventilation using a gamma camera. The 13 half-life Krypton-18m was used; generated from cyclotron produced Rubidium-81 - 4.7 hours half-life. The low radiation dose associated with the inhalation of $^{81}Kr^{m}$ and the clinical potential of the procedure made this methodology particularly suitable for consideration in the contract. During the project, specific clinical applications of this pulmonary ventilation method, were considered, and coupled with the existing method for monitoring pulmonary blood flow using Technecium-99m labelled microspheres. The value of these methods was explored in obstructive lung diseases such as bronchitis and asthma, and where appropriate chest X-ray comparisons were made. This was followed by studies in such

chronic lung diseases as emphysema and pulmonary cancer. The resulting experience of this ventilation method's sensitivity for detecting lung disease, together with the low radiation dose to the patient, directed its use to diagnosing pulmonary problems in children. Experience in the paediatric area grew quickly. A specific use of cyclotron produced nitrogen-13, which has a ten minute half-life, was also found in children. When used to measure pulmonary blood flow/ventilation, a sensitive means emerged for identifying pulmonary hypertension in cases of cardiac shunts. The use of $^{81}Kr^{m}$ requires the availability of cyclotron produced rubidium-81. Hence the documented value of the ventilation methods stimulated the search for a tracer which was more convincingly available. For this, the inhalation of aerosols containing mini microspheres labelled with Technecium-99m or Indium-113m was considered. The patterns of aerosol deposition in the lung were compared to those of Krypton-81m. It was found that with careful consideration to particle size, and in the absence of airways disease, that aerosols provide an acceptable alternative for studying pulmonary ventilation. In mixed pulmonary disease $^{81}Kr^{m}$ was to be preferred. In their own right the particular pattern of deposition and the time course of pulmonary clearance of aerosols was found to be sensitive to pathology. Towards the end of the project the use of the gamma camera was extended from a two dimensional display readout to one of transaxial tomography. This was achieved by rotating a camera around the chest during data collection.

The phenomenon of pulmonary oedema is frequently associated with cardiac disease, hence attention was given to the use of tracers to measure the accumulation of lung water. Water labelled with the 2.1 minute half-life oxygen-15 was used, as well as measurements of protein leakage rates into the extravascular lung space. Although encouraging results were obtained, the techniques were found to be limiting with respect to the availability of tracers and the high measuring precision needed for following the time course of leakage.

In the latter phase of the project, attention was focussed on the application of a positron emission tomograph (PET) for measuring pulmonary parameters. The success of previous gamma ray camera work, made it difficult to identify areas where PET technology may have a role in pulmonary studies. An extension to the earlier lung water space measurements was developed. This involved measuring regional lung density with a PET transmission scan followed by a PET emission scan of pulmonary blood

volume using red blood cells labelled with ^{11}CO. The subtraction of the blood from the density scans provided an absolute quantitative measurement of the extravascular lung space. At the end of the contract period clinical studies were being initiated with this method.

A new era of pulmonary studies with radioisotopes was explored. This involved moving from the measurement of such physical parameters as flow and volume, to that of the lung's metabolism itself. In this first in vivo study of the metabolic lung, Carbon-11 labelled palmitate was administered to animals and the lungs scanned tomographically, in an attempt to identify the sequestration of tracer into the lung as a marker of pulmonary surfactant production. The preliminary signals recorded were disappointingly low, and alternative pulmonary metabolism precursors will need to be considered.

Radioisotopic studies of regional pulmonary function represent one of the most common and unique uses of radionuclides in man[1]. The results of this project provide a closer understanding of the benefit of the radiation load and provide some foresight as to where these techniques may develop in the future. It has not been possible to fully cover a comparison of these new methods with the information affected by radiological examination. A detailed analysis of this may be of relevance to the Euratom organisation if their new pulmonary isotopic methods become universily accepted.

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Project No. 3.

Head of Project: Professor J.P. Shillingford/Dr. A.P. Selwyn.

Scientific Staff: Professor R.E. Steiner, Dr. R.M. Allan,
Dr. J.P. Lavender, Dr. V.W. Pike,
Dr. A. Kivissaro, Mr. T.A. Pratt,
Dr. K.M. Fox, Mr. G.R. Forse,
Dr. C.G. MacArthur, Mr. P.L. Horlock.

Title of Project:

Exposure to Radiation in Cardiac Diagnostic Procedures.

This project has concentrated on the development and clinical application of methods to measure regional cardiac muscle blood flow and metabolism. Such measurements are required to help identify the presence of the physiological disturbances that underly coronary artery disease. In addition to using these methods to describe the nature and extent of the physiological lesions, the opportunity to follow the efficiency of treatment at a level of tissue function, is highly desirable. In the context of these questions the diagnostic value of the tests developed are presented and may be related to the associated radiation doses to the patient.

At the initiation of the project a technique had just been established at Hammersmith to monitor regional blood flow to the cardiac muscle. This is a relatively low radiation dose procedure involving Krypton-81m which has a 13 second half life. The method required this tracer to be introduced continuously, in solution form, through an arterial catheter which irrigated the coronary sinus. While patients were being catheterised for angiographic studies of the heart, the opportunity was taken to apply the myocardial Krypton-81m blood flow procedure. A mobile gamma camera system was developed which allowed images of regional myocardial perfusion to be recorded at the time of coronary angiography. The short half life of the tracer allowed repeat measurements of myocardial perfusion to be made, and hence sequential changes of regional flow during exercise or pacing could be examined. The accuracy of this technique for such studies was established in animals prior to and in parallel to the clinical work. At angiography, coronary artery narrowing is seen. The blood flow method was used to assess the physiological significance of this by mapping out the flow in the capillary bed, fed by those arteries. The results of this comparison are highly relevant in that appreciable radiation doses are associated with this routinely applied diagnostic radiological procedures. It was found that coronary artery stenosis was often a poor guide for assessing the

extent of the physiological disturbances.

By following the response during cardiac pacing, it was found that it is possible to discriminate between permanent and reversible disturbances of regional myocardial blood flow. This had an immediate significance with respect to the assessment of patients who may benefit from coronary artery by-pass surgery. The use of the Krypton-81m method was extended to the operating theatre. There it was possible to introduce the tracer directly into the by-pass graft, and so assess the territory supplied by the anastomosis. Post-operative angiography and blood flow studies again helped to assess the eventual physiological outcome of the operation. These measurements confirmed that myocardial perfusion can be returned to the normal level by this surgical procedure.

In many hospitals the myocardial scanning agent Thallium 201, given intravenously, is used to assess focal defects in the heart muscle. The opportunity was taken to assess how well this test could depict focal blood flow defects in the heart muscle. An animal model of coronary artery ischaemia was used, and it was shown that Thallium 201 only depicts focal reductions in flow provided tissue metabolism is also effected. This has special relevance to the value of the radiation loads which result from the frequent use of Thallium 201 in diagnostic cardiology. By comparing the blood flow changes seen using Krypton-81m with ECG and blood enzyme changes, it was concluded that the simple non-radiation tests are sensitive for detecting myocardial ischaemia. Near the end of the project the rotating gamma ray camera was used to tomographically delineate the distribution of Krypton-81m and hence blood flow in the human heart. This in turn provided a higher detection accuracy for a given patient dose. Following the installation of the positron emission tomograph in 1979, attention was focussed on the use of positron emitting isotopes for non-invasively monitoring regional myocardial function. Using this new technology to provide transaxial tomographic slices of the isotope's distribution within the cardiac tissue, three new isotopic methods were developed.

The continuous intravenous infusion of the 1.3 minute half-life Rubidium-82 provided myocardial tomograms of regional cation uptake/blood flow. Carbon-11 labelled acetate was used to study regional energy metabolism in the heart and the continuous inhalation of oxygen-15 labelled carbon dioxide provided, for the first time, a non-invasive means for measuring regional myocardial blood flow. The methods have

been applied to patients with both coronary artery disease and cardiomyopathy. The effect of exercise on regional function could also be followed. In a short space of time it became clear that new fundamental information can be obtained with these tracer techniques and their associated radiation loads. Although these tests are currently confined to relatively few Medical Centres, the prevelance of heart disease indicates that they may become more widely established in cardiac medicine. The community can now be aware of the information: radiation cost which underlies these methods. It was not possible to comprehensively cover a corresponding analysis for X-ray radiological cardiac procedure. This could be a subject for further study within the community.

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Project No. 5.

Head of Project: Dr. C.G. McKenzie.

Scientific Staff: Dr. G.L. Lenzi, Mr. J.D. Heather,
Dr. A. Pinching, Mr. S. Moss,
Dr. G.R.V. Hughes, Mr. J. Ensell,
Dr. R.S.J. Frackowiak, Mr. J.C. Clark,
Dr. T. Jones, Mr. P.D. Buckingham.

Title of Project:

Exposure to radiation in brain diagnostic procedure.

This project has been concerned with the development and clinical use of radioisotopic methods to measure regional brain blood flow and metabolism. This concentration arises from the need for further information on the cerebral patho-physiologies that underly certain common neurological conditions and how or whether these respond to treatment. During this contract attention has focussed specifically on investigating the regional energy requirements and balance of the diseased brain. This was possible by developing a technique which couples a measurement of tissue blood flow to that of oxygen consumption. The radioisotope oxygen-15 has been used for these measurements and having a half-life of 2.1 minutes needs to be produced on line by the cyclotron. It is the longest lived gamma ray emitting form of oxygen. Hence it is essentially the only nuclide that can be used to measure the basic tissue phenomenon of oxygen consumption. The procedure developed consists of piping the isotope from the cyclotron to the clinical investigation area. Here it is inhaled continually by the patient to produce a steady state concentration of tracer within the tissue. At this point the distribution of the isotope within the brain is monitored with radiation detectors. Two separate inhalation procedures are involved: the inhalation of oxygen-15 labelled molecular oxygen to study oxygen utilisation, and oxygen-15 labelled carbon dioxide to study blood flow. The information which this procedure can provide may be related to the associated patient radiation absorbed dose.

The initial studies with oxygen-15 involved the use of a conventional gamma ray camera to measure the cerebral distributions. The data provided by the instrument is at best semi-quantitative. This results from the two dimensional recording of a complex three dimensional distribution of tracer. Nevertheless, the read out from this camera was used as a means of exploring whether or not this tracer approach offers sensitivity for identifying and describing focal cerebral disease. A wide range of neurological patients were studied, Brain Tumours, Dementia,

Cerebral Vascular Disease, Parkinsonism, and generalised vasculitic disease, together with a normal population. In all cases focal disturbances and uncoupling of flow and metabolism were detected. In some instances the method was used routinely to follow the time course of patients undergoing treatment. One particular example of this was to assess the efficiency of by-pass surgery for the treatment of cerebral ischaemia. This application high-lighted the differences between radiological and isotopic measurements to assess focal cerebral defects. In total some 640 patient measurements of flow and metabolism were carried out in this initial qualitative phase of the project. The results from this study provided one of the main cases for the installation of a positron emission tomograph in the Unit in 1979. The use of this Scanner immediately advanced the accuracy of the cerebral measurements on two fronts. The tomographic presentation of data removed the signal superimposition, and the ability to quantitate tracer concentration allowed the signals to be processed to provide absolute values of tissue blood flow and oxygen consumption rates. The range of these physiological parameters for normal subjects was established and clinical studies initiated. A total of 230 neurological patients were examined tomographically with principle emphasis on stroke, dementia and tumour cases. A number of new findings emerged from this data which have helped define the underlying patho-physiologies. In stroke cases it was found that within the lesion, there was very often an excess of blood flow relative to metabolic demand, and the depressed metabolic rate was seen to recover in time, in a significant number of cases. These results have a direct bearing on the therapy of this disease. The value of drugs; intended to improve flow, is in doubt in approximately 80% of the cases, and that concern needs to be given principly to specifically distinguish the nature of the physiological lesion prior to prescribing therapy. In pre-senile dementia, both blood flow and metabolism are equally depressed which again points to the defect being principally metabolic, rather than ischaemic. In tumours the results put the phenomenon of neoplasm anoxia in question. Although the findings are somewhat divorced from a current routine clinical diagnostic procedure, it is important to underline their relevance to the understanding and treatment of common neurological diseases. The results indicate that there now exists objective ways of assessing treatment, which cannot be obtained by such methods as X-ray transmission. This still preliminary data is stimulating many centres in Europe to establish

similar facilities. This in turn has direct relevance to the clinical radiation loads which will arise from this means for monitoring. It is envisaged that for the foreseeable future, the actual number of patients involved, will be small.

The community is now prepared for the growing diagnostic interest in this source of radiation exposure to patients undergoing Neurological examination. In turn a closer examination of the value of radiological x-ray patient doses; an aspect not fully covered in the project, may become relevant to the concern of the Euratom organisation.

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13. Frackowiak R.S.J., Lenzi G.L., Jones T., Heather J.D.: Quantitative measurement of regional cerebral blood flow and oxygen metabolism in man using ^{15}O and positron emission tomography : Theory, procedure and normal values. J. Comp. Asst. Tomo. 4 (6), 727-736, 1980.

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Project No. 6.

Head of Project: Mr. T. Jones.

Scientific Staff: Mr. G.R. Forse, Dr. M. Myers,
Mr. J.D. Heather, Dr. J.E. Bateman,
Mr. C.G. Rhodes, Dr. J.F. Connelly.

Reduction of radiation exposure through optimal detector design.

In considering the optimal instrumentation for detecting the in-vivo distribution of radioisotopes, principle consideration has to be given to both accuracy and sensitivity of the devices being used. In effect a benefit:cost balance is evident. The physical specifications of a measurement needs to be related to the cost of achieving that measurement in terms of the radiation dose needed to obtain the required statistical accuracy.

During the period of the contract such a consideration was applied to two categories of instrument. This arises from the use in our institute of isotopes that emit either single photons or paired annhilation photons (following positron decay)- see projects 2,3 and 5. The optimal means for detecting the 511 KeV annhilation photons emitted from the body following positron capture represented the greatest consideration in this project. This arose since the technology for detecting single photon emitters has already been well established, following the widespread use of Technecium-99m in the field of diagnostic Nuclear Medicine. The use of positron emitters however, is confined to relatively few centres, and hence there has been less stimulus for their technical advance.

At the beginning of the project the single photon Krypton-81m and Technecium-99m work (projects 2 and 3), was adequately covered by the availability of state of the art wide field of view Anger Gamma ray cameras. Although they were being used for detecting 511 KeV annhilation photons, this was only possible at the cost of poor sensitivity which arises from the need for thick lead collimators, and the thinness of the camera detecting crystals. At the early stages of the project, an analysis of the positron detection field indicated that developments, principally in the United States, of polygon arrays of detectors, operating in coincidence around a single transaxial plane, were the most promising with respect to spatial detection accuracy. It was clear, however, that in these instruments only a small solid angle of detection was used. Thus in parallel to following the developments of these machines, it was decided to support the construction of an area positron camera in the United Kingdom. The rationale for this lay in seeking to

use higher detector solid angles than the single slice machines provided. Thus the instruments to be used for positron isotope detection advanced on two fronts:-

a). A commercial single slice positron emission computerised axial tomograph ECAT, was installed in the institute. This machine was extensively tested with respect to its physical performance. In conclusion it was found to be the most accurate device available for measuring the regional concentration of tracer within the tissue of man. This finding is based not only on its tomographic read out, but also on the linearity of response and the ability to correct absolutely for tissue attenuation of the emitted photons. From the accuracy point of view, this machine has currently produced the greatest accuracy benefit relative to the patient absorbed doses. The device is an instrument which has transformed the concept of Nuclear Medicine from what to date has been mainly a qualitative imaging discipline, to one of precise measurements of tissue radioactivity. In turn this new dimension of precision allows quantitative values of tissue function to be produced. This salient point is underlined in the reports on projects 2,3 and 5. Given the precision of this basic design and the usefulness of the clinical data that can now be produced, there exists a strong incentive for commercial manufacturers to improve the sensitivity of this generation of machines even further. Not least in this is the need for higher sensitivity and hence a reduction of patient dose. This can be achieved by increasing the number of planes of data that can be collected simultaneously. This point has a direct bearing on machines that are to be installed in European centres in the near future.

b) The area positron camera developed during the period of the contract consisted of stacks of multiwire proportional chambers. This detection principle is based on the extensive technology that has been developed in Europe in connection with the high energy Nuclear Physics projects. A camera 30 cm by 30 cm in area was constructed, and by the end of the project period, data was being collected both from physical phantoms and animals. The spatial resolution is three times better than the current single slice tomograms. The intrinsic sensitivity of the proportional chamber is significantly less than that for the sodium iodide detectors used in the ECAT. However it was found that the area positron camera has a sensitivity comparable to that of the ECAT, when viewing heart or brain phantoms. This arises from the greater solid

angle of detection subtended by the area machine. The problems of extracting truly quantitative data from the area positron camera has yet to be fully solved. Not least are the problems of a background noise due to scattered coincidence events.

The next stage in the area camera development could be to produce a polygon array of multiwire chambers, which completely enclose the patient and so maximise the solid angle for detection. The indications are that to increase sensitivity, and hence reduce patient dose while still maintaining quantitative accuracy, the near future development will be directed towards multi planes of single slice detector arrays. Developments with single photon Gamma ray cameras consisted of mobilising a small device to collect data at angiography and surgery. In this way the isotope is administered selectively into the organ of interest. Hence radiation doses to the other body tissues was minimised. The use of a rotating X-ray camera has shown that tomograms can be obtained that are linear in their response with tissue activity concentration. This in effect extracts more accurate information for a given patient dose. The limits on the absolute quantitative accuracy for this have yet to be established.

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3. Bateman J.E., Connolly, J.F., Stephenson R., and Flesher A.C.: The development of the Rutherford Laboratory MWPC Positron Camera (1980). Nucl. Inst. & Methods. 176. p. 83-88.

Contractor of the Commission :

School of Nuclear Medicine of the University of Pisa

Contract n. 228-76-7 BIO I

Head of Research Group: Prof. Luigi Donato

General theme of the contract:

Exposure to radiation from medical diagnostic procedures.

Title of project n.1:

Assessment of current radiation load from medical exposure.

Head of the project and scientific co-workers:

Riccardo Guzzardi, Riccardo Bellina, Maurizio Mey, Ubaldo Bottigli

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1. Report of the activity during the 1980

a) Assessment of radiological procedures

With the aim of assessing the information content, image quality and the patient radiation dose in cardiovascular procedures, the technique of x-ray ventriculography using continuos infusion of a contrast medium has been analyzed. To this purpose, the amount of contrast medium in the left ventricle, has been calculated through an analytical simulation of the involved dynamic process (continuos infusion in the contractile chamber). This to evaluate the quality of the information content in the image and to assess the radiation dose to the patient with respect to the time necessary to get the diagnostic information. The conclusion of this work was that there is an usual and not justified extra irradiation of the patient non associated with the production of reliable and significant information.

b) Patient dosimetry in Nuclear Medicine Investigations

This work has continued both on the side of specific evaluation of patient dosimetry using new diagnostic procedures (the microspheres for heart studies, tagged with different radioisotopes both gamma or positron emitters) and in that of completing the collection of dosimetric data from any in vivo Nuclear Medicine procedure.

This last work, particularly, will come out as a book published by the Commission of the European Communities (Harwood).

2. Results of the project from the start up (1976)

The activity of this project has been almost completely devoted on the methodological side of both development of techniques for the evaluation of the patient radiation load and assessment of the imaging procedures. Specially on the side of the dosimetric methodologies, the main results have been:

a) the development of a software program for the calculation of the radiation dose to the patient organs when new procedures are introduced in Nuclear Medicine investigations;

b) the collection of the dosimetric data, regarding the in vivo Nuclear Medicine procedures. This information will appear as a book published by the EEC.

c) The development of an experimental methodology for internal dose evaluation using the Rando Phantom and TLD dosimeters. (This methodology has been successfully applied to the Compton Tomography of the lung).

On the side of the assessment of the imaging techniques, the main results have been:

a) comparative evaluation of the performance achieved and radiation load using internal gamma sources, trasmitted x-ray and ultrasounds in the investigation of the cardiac function.

b) Comparative evaluation of the new methodology of Compton Scattering Tomography, for study of the lung density, with other methodologies as the Blurring Tomography.

c) Assessment of the x-ray ventriculography in terms of patients radiation load and information content.

Publications

GUZZARDI R., MEY M., PISTOLESI M., SOLFANELLI S., GIUNTINI C.: Tomography 90° Compton scattering. J. Nucl. Med. All. Sci. 21, 72, 1977.

BELLINA R., GUZZARDI R.: CAMIRD III: An up to date version of the CAMIRD III package for internal dosimetry. December 1977, CRN Clinical Physiology Laboratory, Pisa.

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Title of the project No. 2:

Exposure to radiations in pulmonary diagnostic procedures.

Head of the project and scientific co-workers:

Carlo Giuntini, Carlo Marini, Giorgio Di Ricco, Giuseppe Pistelli, Anna Maria Santolicandro, Paolo Paoletti, Massimo Pistolesi, Massimo Miniati, Giuseppe Maltinti.

90°Compton scattering (COSCAT) with an external gamma-ray source versus chest x-ray tomography (CXRT)

During 1980 the technic has been applied in 33 patients affected by pulmonary edema of cardiac (hydrostatic) or injury (increased permeability) origin. The clinical usefulness of COSCAT was tested versus chest x-ray, (the most used clinical method to assess pulmonary edema): the radiation load of the two technics is comparable. In cardiac patients both methods reveal a predominantly central perihilar distribution of increased lung density, whereas in patients with injury pulmonary edema a patchy, more peripheral and less gravity dependent distribution was observed. COSCAT is more accurate in defining the above abnormalities and more sensitive in detecting density changes with time. Moreover, scissural and subpleural effusions not clearly evidenced by Chest x-ray, were visualized by COSCAT. In cardiac patients, during pulmonary edema formation or regression, hilar size and perihilar density changes not observed by sequential Chest x-ray were evidenced in COSCAT performed at the same time. The latter may thus be considered an adjunctive diagnostic tool for non invasive detection and monitoring of pulmonary edema, while may also permit differentiation between cardiogenic and injury pulmonary edema, pointing out regional differences in accumulation and reabsorptions patterns in the two different types of pulmonary edema.

In order to obtain absolute values of lung density, in 1980 the computer correction for physical attenuation of both primary and scattered gamma rays in chest tissues, has been further developed. Gamma camera imaging signals recorded on 35 mm film are digitized by a computer controlled flying spot system; several processing steps are then executed. Colour images with counting levels proportional to the absolute value of scanned tissue density are finally displayed on TV monitor. Preliminary application of this computer correction demonstrates the possibility of improving the diagnostic capabilities of the technic. Further development of the computer algorithms will permit the reconstruction of tranverse tomographic planes from the informations obtained by the sagittal and frontal planes.

During the three years of the project the technic has been applied in 68 patients with various disorders that alter diffusely or focally lung density. In many of these patients, the imaging technics commonly used can not give clinical information about lung density comparable to that of COSCAT. In particular, conventional CXRT did not offer the same density discrimination in studying lung parenchyma. Indeed, the tomographic effects of CXRT is obtained blurring the structures at different depth by means of relative motion of x-rays source and detector. COSCAT is obtained with stationarity of both gamma-rays source and detector. Furthermore, the field of view of CXRT is smaller with respect to that of COSCAT. The skin dose to the patient in conventional CXRT has been calculated in 470 mR for one tomogram of the chest (Harle T. e al., Am. J. Roentgenol., 124: 353, 1975). We calculated a total absorbed dose of 93 mR for a COSCAT.

Assessment of radiation exposure and significance in the diagnosis and treatment of obstructive airways disease of inhaled 99mTc-minimicrospheres (99mTc-HAMM).

The purpose of this project was to investigate the aerosol deposition and clearance in airways of normal people and of patients with lung disorders, in order to allow computation of radiation exposure due to inhalation of active aerosols. As a matter of fact, very few informations are available on the fate of inhaled aerosols in people with lung disease. The high incidence of lung disorders in most european countries makes it useful to have some references for more accurate assessment of radiation exposure due to radionuclide entering the body by inhalation.

The pattern of deposition of 99mTc-HAMM, a monodisperse aerosol of albumin particles having an aerodynamic diameter of 0.75 µm and a geometric standard deviation of 1.19, was revealed with a large field camera (Toshiba GCA2) soon after nebulization and 4 hours later. We studied more than 200 patients affected by obstructive lung disorders.

The pattern of deposition in normal subjects is characterized by homogeneous distribution of radioactivity and by gradient from base to apex. Most of the inhaled particles deposit by sedimentation in airways

peripheral to the 16th generation. In patients with asthma the images show that most of the deposition is in central airways. If a reduction in caliber of the first 10 generations is assumed, deposition of particles may increase to up 100 fold the normal, according to the degree of obstruction. In patients with chronic bronchitis or emphysema the images show roundish spots of particles deposition; the spots with diameter of few centimeter are located in the peripheral regions of the lungs, their shape and position being poorly influenced by the bronchial clearance. If we assume a reduction in caliber of the peripheral airways the deposition by impaction in this region increases only of a small amount. Therefore the marked uneveness of this pattern is due to the uneveness of air flow distribution consequent to the distruction or reduction in caliber of peripheral airways.

These different patterns of deposition permit to identify the site of prevalent broncoconstriction in lung disease.

From the images obtained we computed the relative amount of total and regional deposition and the clearance efficiency for particles having the same dimensions of HAMM.

The main factors influencing particles deposition are particles size and bronchial caliber (fig. 1).

In normal subjects the radiation exposure to the whole lung, considered like a sphere of unity density and with 20 cm diameter, resulted 0.402 mrad/mCi inhaled by the patient, details of the calculations have been reported in 1978.

Figures of regional deposition and of clearance are reported in Table I. In column 2 and 3 are reported the reduction in caliber assumed for computation of the total deposition (column 4) in per cent of inhaled and the relative deposition in central (column 5 and 6) and peripheral airways (column 7). The experimental values of activity cleared in 4 hours, obtained on the late images, are reported in column 8 (mean values of the determinations in each group of subjects). These last figures are higher than the computed ones (column 6+7) for the presence of deposition in the mounth and in the digestive trac.

During the 4 years of this project 99mTc-HAMM inhalation has showed to be an useful technique to reveal the site of bronchial constriction and the degree of impairment of bulk flow ventilation. This technique is a valid diagnostic mean to differentiate the various obstructive lung disorders and to follow up treatment effect.

Moreover the data on aerosol retention, in airways of people with obstructive lung disease, are useful references to calculate radiation exposure to inhaled radioactive aerosols for people with obstructive lung disease.

DETERMINANTS OF PARTICLES DEPOSITION IN AIRWAYS AIRWAYS CALIBER, AIR FLOW AND PARTICLES SIZE

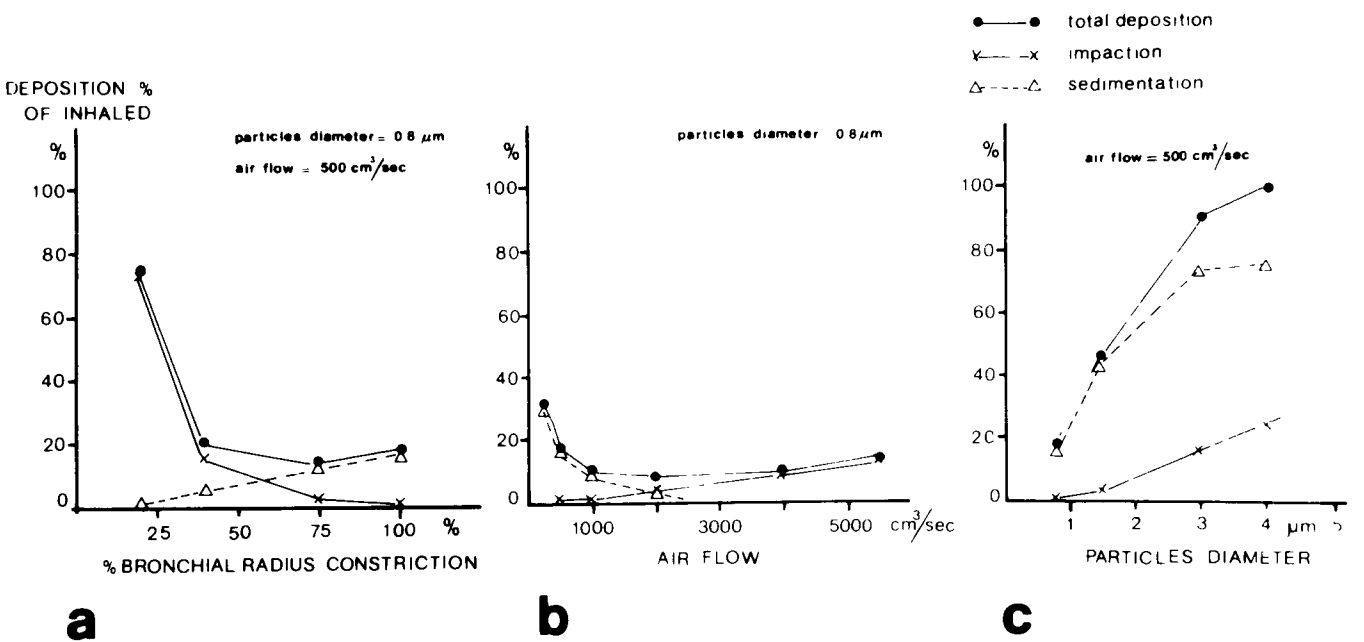

TABLE I

ASSESSMENT OF RADIATION EXPOSURE AND SIGNIFICANCE IN THE DIAGNOSIS AND TREATMENT OF OBSTRUCTIVE AIRWAY DISEASE OF INHALED ^{99m}Tc-MINIMICROSPHERES (^{99m}Tc-HAMM)

Simulated airways condition	Radius	Cross Section	Particles deposition				Activity cleared in 4 hours	Deposition per unit bronchial mucosa	
			Total	0 - 10th generatic		0-18th generation			
				a	b				
	% of normal	% of normal	% of inhaled	% of inhaled	% of deposited	% of deposited	% of initial	Total	0 - 10 generation
"Normal"	normal	normal	17	0.9	5.3	12.3	24.8	1	1
"Asthma"	80	-	13.6	1.5	11.0			0.7	2.5
	60	-	18.7	3.6	19.3			1	6.1
	20	-	69.9	60.5	86.6	87	86	-	82
"Chronic Bronchitis and Emphysema"	-	50	11.9	0.9	7.5			1.4	-
	-	25	9.6	0.9	9.3	20	66.3	2.3	-

Diagnostic relevance in relation to radiation dose to individual patient for pulmonary arteriography and 131-I-AMA lung scanning.

To calculate the x-rays doses delivered to patients during a pulmonary arteriography it is necessary to account for the body size, its position in radiological field and the distance from the tube to the skin when the experimental examination is carried out.

A reliable evaluation of the delivered dose is obtained by estimating changes of the main technical parameters in relation to the unwanted radiations delivered to thyroid and gonads.

To estimate the delivered absolute dose we measured firt the power supply of our x-rays tubes to verify the conformity with requested data and then we applied TLD to the x-rays beam exit; in this way we obtained a calibration curve relating the read-out of TLD 100 to radiations measurements carried out by two ionization chambers (Jonex dose/dosemeter 2500/3 with a 0,6 cc chamber 2505/3A and 600 cc thin window chamber 2511/3).

An "average man random phantom" was used to performe a shame pulmonary arteriography and to measure the relative delivered dose.

X-rays films were inserted within phantom sections at thyroid, lung and feminine gonads levels; for the masculine gonads films were placed on the skin. We used X- omat Kodak films automatically developed by Agfa G138 developper and Agfa G334 fixer at 33°C for 90".

The pictures, transferred an a 24x36 mm film, were read-out by the SADAF system. In this way pictures were turned into digital form: they were sampled by 24 micron steps and the obtained values were quantified an 64 density levels. The system includes: a) flying-spot analyzer of films b) PDP8/1 computer c) TV monitor and d) Tape recording of data.

Data analysis has been performed by VIDAR system consisting in a number of programs linked together and adapted for a MP21MX computer which provided: a) Picture hystograms plotting b) Noise level cut-off c) picture reproduction into the gray range d) stereograms and isodose curves plotting. The X-rays dose distribution is homogeneous at gonads levels, not at thyroid and lungs levels.

To explain this finding we must consider the chest as a source of diffuse radiations; because gonads area is rather far from the examined area, it receives a radiation dose changing with the square of the distance as it was shown by a direct measurements.

Measurement of the absolute dose performed along the phantom anteroposterior axis in the areas with x-rays films have shown at gonads level a difference of about 5% between the directly exposed and more posterior areas; this difference observed in the section through the thyroid was about 20% and became much larger in the chest level sections. See table I.

TABLE I

Pulmonary arteriography in a patient weighting 70 Kg - A-P projection
(17 radiograms - 76 KV - 60 m AS)

	gonads	50 ± 2.5	mR
	thyroid	240 ± 7.5	mR
Chest	anterior skin surface	20	R
	depth 12.5 cm	680 ± 135	mR
	posterior skin surface	136 ± 27	mR

Owing to increasing number of diagnostic and therapeutic procedures available in radiological field at the present time, there is a great deal of research not only about the development of new dosimetric techniques but also about the applications of these new techniques to particular target organs, in different populations of different age.

In the few dosimetric studies available up to now about the pulmonary angiography there is a large scattering of values obtained in patient during this diagnostic procedure (J.G. Gough et al., Br. J. Radiol. 41:508, 1968; F.C. Rueter, Circulation 58 (1): 1349, July 1978).

Radiations dose delivered to patients during pulmonary scintigraphy as been calculated according to Furth et al., J. Nucl. Med. 6: 506, 1965.

Following their measurements the total dose to lungs is 0.64 rads for 100 μCi of 131-I-AMA delivered. Hence, a patient weighting 70 Kg receives, during pulmonary scintigraphy by 131-I-AMA (about 420 μCi), about 2.7 Rads to the lungs.

Similar values for the radiation dose delivered to the lungs are reported from the International Atomic Energy (G.J. Hine and R.E. Johnston, J. Nucl. Med. 11: 468, 1970) and they came out by about 8% higher the mean values reported in literature until 1970 (G.J. Hine and R.E. Johnston, J. Nucl. Med. 11: 468, 1970).

From the study by Furth et al. the radiation dose to the gonads results 0.17 Rads in a patient weighting 70 kg (without significative difference between testes and ovaries). We considered, as previous reported authors, the effect on the kinetics of body disposal of 131-I of thyroid blocking doses of "Lugol" administered to the patient prior to 131-I-AMA injection and in following days.

We studied pulmonary scintigraphy in the diagnosis of pulmonary embolism (C. Giuntini et al., Boll. Soc. It. Cardiol. 17: 929, 1972; A. Santolicandro et al., Abstract Book, I, 481, 1976, 7th Europ. Congress of Cardiology, Amsterdam, June 20-25, 1976); in this disease arteriography and scintigraphy were both applied and compared each other (C. Marini et al., J. Nucl. Med. All. Sci. 22: 79, 1978.

Data provided by our patients show that pulmonary scintigraphy by 131-I-AMA is the most specific and accurate tool in the diagnosis and follow-up of pulmonary embolism. Total lung dose, compared to pulmonary angiography is about the same, whereas gonads dose is somewhat higher and skin irradiation much less.

By means of scintigraphy it is possible to follow the disease in its evolution also when the angiography fails to visualize the embolic material fragmented and dispersed into the pheripheral vessels of the lung.

Furthermore the angiographic technique may be dangerous in very ill patients, in whom pulmonary scintigraphy can be always performed without risk.

In our experience pulmonary lung scans of patients suffering of chronic obstructive lung disease do not follow a segmental pattern of perfusion defects that is on the contrary evident in patients affected by pulmonary embolism. We believe that pulmonary embolism can be clearly distinguished from chronic pulmonary lung diseases and, in our experience, false positive diagnosis is not a problem at 131-I-AMA lung scan (C. Marini et al., Frontiers of Nuclear Medicine edited by W. Horst H.N., Wagner Jr. J. W. Buchanan. Springer Verlag Berlin Heidelberg 1980,242 255).

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Title of project n. 3:

Exposure to radiation in cardiac diagnostic procedures.

Head of the project and scientific co-workers:

Attilio Maseri, Mario Mariani, Ignazio Simonetti, Nilda Uthurralt, Sandra Giusti, Rita Mariotti

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1. Radiation production during routine coronary angiographic diagnostic investigation

In order to obtain information on radiation exposure during routine coronary angiographic studies we collected the data employed in 1980 for 163 consecutive cases. All had selective right and left coronary cineangiography, 6 to 14 injections, left cineventricolography, 1 to 3 injections; in 22 patients also abdomino-iliac cineangiography was performed, 1 injection. 158 studies were performed using Judkins Technique, while in 5 cases Sones' technique was employed.

Two physicians were in the catheterization room during fluoroscopy and coronary arteriography; one was present during aortography (for the displacement of the table). No-one was inside the room during ventriculography. No other personnel was involved within the catheterization room during these procedures. The operators were protected by standard lead aprons 0,155 mm thick.

The radiologic conditions used were 0.5-2.5 mA and 70-110 Kv; cine-coronary angiograms were obtained by pulsed technique using 2-6 msec pulses of 400-500 mA and 60-90 Kv, at 50 frames/sec for ventriculography and 30 frames/sec for coronary angiography and aortography. The duration of the recording ranged between 4 and 8 seconds.

For each case the following physical data were collected: 1) patient's age, sex, weight, height, trunk length, frontal half-length, sagittal half-length, beam-gonads distance, focus-skin distance; 2) focus-distance for the operator's gonads and hands, during the various phases of the investigation (Table I and II).

In Table III are listed the ranges of the patient absorbed radiation doses in the various diagnostic procedures. For each procedure both fluoroscopy and cineangiography have been considered. In practice each diagnostic workup entails a combination of ventriculography plus coronary angiography $\pm$ abdominal aorta angiography (1+2, or 1+2+3). Organ doses have been calculated by the computerized procedure described by M. Rasenstein ("Organ doses in diagnostic radiology", HEW Publication - FDA, 76-8030). As shown in the Table I, gonadal doses are very low indeed, reaching measurable levels only for the ovaries in abdominal aorta imaging. Bone marrow exposure may attain significant levels in the case of coronary angiograms. Total body dose is also not negligible for coronary and ventricular angiography. Exposure to operators has been evaluated and listed in Table IV. Operators always use lead aprons 0.155 mm thick, therefore the gonad dose is negligible, while significant can be (depending on the duration of the procedure) the exposure to the hands.

2. Radioisotopic dose during myocardial perfusion studies using 201-Thallium scintigraphy.

The procedure entails the intravenous injection of 1 mc of 201-Tl and subsequent scintigraphy in multiple projection of the patient. One doctor

and one technician an involved in the procedure. In 1980 a total of 48 studies on 35 patients were performed, during acute myocardial ischemia; in 13 patients the study was repeated a week later, in the absence of symptoms.

The main physical characteristic of the patients, and the duration of the various phases of the study are listed in table IV. Resulting absorbed dose to the patient is also listed in Table V.

TABLE I

Average patients and operational characteristics

N.patients	Age (years)	Weight (kg)	Height (cm)	Trunk length (cm)	Frontal Half-length (cm)	Sagittal Half-length (cm)	Beam-Gonads distance(cm)	Focus-skin distance(cm)
163	57±20	64±16	174±22	64±9	20±5	14±4	41±12	67±9

TABLE II

Average operator/beam distances for cardiac diagnostic provedures

	BEAM-GONADS DISTANCE cm	BEAM-HANDS DISTANCE cm	BEAM-GONADS DISTANCE cm	BEAM-HANDS DISTANCE cm
Fluoroscopy	61 ± 18	38 ± 12	89 ± 23	58 ± 35
Coronary cineangio-graphy	75 ± 12	40 ± 15	140 ± 41	130 ± 35
Ventriculo-graphy	-	-	-	-
Aortography	98 ± 19	60 ± 7.5	-	-
	FIRST OPERATOR		SECOND OPERATOR	

TABLE III

Ranges of absorbed doses from cardiac diagnostic procedures

		MAS	EET sec	ABSORBED DOSE (mrad) OVARIES	TESTES	THYROID	BONE MARROW	TOTAL BODY
1. CORONARY IMAGING	FLUOROSCOPY	180-3100	-	<2	negl.	0.8-16.6	7.9-149.6	27.9-391.0
	CINE ANGIOGRAPHY	-	4.4-21.6	1.6-5.0	negl.	18.2-56.1	163.2-501.7	428.8-1318.1
2. VENTRICULAR IMAGING	FLUOROSCOPY	40-1080	-	<1	negl.	0.2-5.3	1.7-47.5	4.6-124.2
	CINE VENTRICULOGRAPHY	-	1.8-5.1	<2	negl.	7.4-18.9	66.5-169.7	174.8-445.9
3. AORTIC IMAGING	FLUOROSCOPY	15-187	-	negl.	negl.	<1	0.6-8.2	1.7-21.5
	CINE AORTOGRAPHY	-	1.5-2.4	74.7-116.1	<2	negl.	38.7-60.1	156.9-243.6

MAS = Milliampères/sec
EET = Exposure time per frame x number of frames
negl.= negligible <0.01 mrad.

TABLE IV

Operator exposure during routine diagnostic procedures (mR).

		FIRST OPERATOR		SECOND OPERATOR	
		GONADS	HANDS	GONADS	HANDS
CORONARY IMAGING	FLUOROSCOPY	negl.	38.4-736.4	negl.	11.9-226.4
	CINEANGIOGRAPHY	negl.	21.3-65.6	negl.	2.4-7.3
VENTRICULAR IMAGING	FLUOROSCOPY	negl.	0.08-233.9	negl.	2.6-71.9
	VENTRICULOGRAPHY	-	-	-	-
AORTIC IMAGING	FLUOROSCOPY	negl.	3.2-40.6	negl.	0.9-12.4
	AORTOGRAPHY	negl.	4.5-6.9	negl.	0.5-0.8

TABLE V

Average patients and operational characteristics. Average absorbed dose during radioisotopic diagnostic procedures.

Sex	Patients	Age yrs	Weight kg	Length cm	TORAX DIMENSION Sagittal cm	Frontal cm
M	40	51±12	65±9	171±10	22±4	46±7
F	8	53± 6	60±10	162±6	20±5	39±7

No. SCINTIGRAMS FOR PATIENTS		12 ± 6
No. SCINTIGRAMS FOR STUDY		7 ± 2
TIME OF STUDY		50 ± 12 min
TIME OF SCINTIGRAM		2 min ± 30 sec
Absorbed dose	TOTAL BODY	0.24 Rads/mCi
	KIDNEYS	0.39 "
	TESTES	0.30 "

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Title of the project No. 4:

Exposure to radiations in renal diagnostic procedures.

Head of the project and scientific co-workers:

Claudio Bianchi, Mario Bonadio, Carlo Donadio, Gianfranco Tramonti, Tommaso Cotronei, Claudio Lensi.

Aprotinin-Tc-99m in new tracer for the study of kidney morphology and function.

Pharmacokinetics of labelled aprotinin were studied in 41 patients. Plasma clearance was about half of the GFR (except in renal failure) and the apparent distribution volume was 14.55 $\pm$ 2.67 SD (per cent of b.w.). The renal accumulation of labelled aprotinin was rapid and symmetrical. In the patients with unilateral renal disease the two kidneys exhibited different renal accumulation curves. Urinary excretion of radioactivity was negligible in the first 2 hours after injection. The comparison between aprotinin-Tc^{99m} and DMSA-Tc^{99m} showed a definitely higher urinary radioactivity with DMSA.

In 63 patients with either uni- or bilateral renal disease and with varying degrees of renal impairment (from normal GFR to advanced renal failure) renal scans were performed by means of aprotinin labelled with I^{131} or Tc^{99m}. Highly satisfactory kidney scans were obtained. A rather low dose of Tc^{99m} is sufficient to obtain satisfactory renal scans with

aprotinin-Tc^{99m}. In fact doses of about 250 mCi of Tc^{99m} supplied diagnostically useful results. In 10 patients with renal failure a comparison was made of the renal scans obtained with aprotinin-Tc^{99m}, DMSA-Tc^{99m}, and chlormerodrin-Hg^{197}. Aprotinin was definitely superior to chlormerodrin in all cases. In the patients with advanced renal failure aprotinin produced slightly better scans than DMSA.

Labelled aprotinin is an excellent agent for renal imaging. It also seems promising for renal functional study.

The most relevant results obtained during the performance of this contract have been: 1) Evaluation of new, noninvasive, methods for the measurement of renal function (total and unilateral), and 2) New tracers for renal imaging.

1. New noninvasive methods for the measurement of renal function

1.1. Bladder cumulative method. It furnishes the direct measurement of total renal clearance (sum of function of both kidneys) by means of a single injection of the tracer and external counting over the bladder, thereby avoiding bladder catheterization and continuous intravenous infusion of the tracer. The bladder cumulative method requires gamma-emitting tracers of sufficient energy for external detection. For the measurement of glomerular filtration rate by the bladder cumulative method a new tracer has been studied. It is the diethylenetriamine-pentaacetic acid (DTPA), labelled with Tc^{99m}. In adult patients the dose used is about 150 Ci.

1.2. Stop-flow method. By this techniques it is possible to measure the unilateral renal function by means of external counting, so avoiding ureteral catheterization. It is based on the measurement by external counting of the increace in radioactivity recorded over each kidney after i.v. administration of DTPA-Tc^{99m} (if the measurement of unilateral GFR is needed), when urine flow to the bladder is stopped.

The use of DTPA-Tc^{99m} by both the above mentioned methods has significantly lowered the radiation doses to the critical organs (gonads, kidneys, bladder) in comparison to the radioiodinated tracers employed in the past (Table I).

TABLE I

	Diatrizoate-I-131 or iothalamate-I^{131}		DPTA-Tc^{99m}	
	mrad/μCi	mrad/test	mrad/μCi	mrad/test
Gonads	0.1 ÷0.4	3 ÷ 12	0.01 - 0.02	2 - 4
Kidneys	≃0.4	≃12	0.05 - 0.3	10 - 60
Bladder			0.4 - 0.6	80 - 120
Thyroid (without Lugol solution)	≃150	≃4500		

2. New tracers for renal imaging

The most widely used tracer for kidney scanning is dimercaptosuccynic acid (DMSA) labelled with Tc^{99m}. Doses of at least 500 Ci are generally used to obtain good renal scans. DMSA and aprotinin, both labelled with Tc^{99m}, have been studied. Aprotinin-Tc^{99m} seems mostly interesting. We have demonstrated that large amounts of aprotinin are accumulated by the animal and human kidney without being neither excreted nor metabolized in the first two hours after i.v. injection. For this reason a rather low dose of Tc^{99m} is sufficient to obtain satisfactory renal scans with aprotinin-Tc^{99m}. In fact doses of about 250 Ci of Tc^{99m} supplied diagnostically useful results, equivalent to those given by higher doses of DMSA-Tc^{99m}. Aprotinin-Tc^{99m} is an excellent agent for renal imaging in man. Although not yet measured, the radiation doses to the patient are probably lower than those of DMSA-Tc^{99m}, due to lower injected dose (see Report 1980).

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Title of the project No. 5:

Exposure to radiations in brain diagnostic procedures.

Head of the project and scientific co-workers:

Ferruccio Fazio, Cesare Fieschi, Marcello Nardini, Cesare Forli, Stefano Solfanelli.

The project has been carried out with the cooperation of the department of Neurology, University of Siena, and the Institute of Neurosurgery, University of Pisa.

From an extensive review of the literature a list has been produced of all radiologic and isotopic procedures of actual or potential use in the assessment of brain disorders. For each procedure estimates of the absorbed radiation dose to the patient have been obtained either from existing data, when available and reliable, or by own calculations. Additionally, schematic data have been collected on the type and quality of information obtained by each procedure. Results are summarized in Table I.

Carotid angiograms are now extensively used for investigating subjects with transitory ischaemic attack (TIA), due to evidence that in these patients a stroke is likely to occur within five years from the onset of symptoms, being life expectancy influenced by treatment and

TABLE I

PROCEDURE	INFORMATION	ABSORBED RADIATION DOSE (millirads)	
SKULL X-RAYS	ANATOMICAL (poor)	EYE	2,000
CAROTID ANGIOGRAPHY	ANATOMICAL (high)	EYE	10,000
AIR ENCEPHALOGRAPHY	ANATOMICAL (fair-special purposes)	EYE	10,000
X-RAY TRANSMISSION	ANATOMICAL (high)	EYE	2,000
^{99m}Tc BRAIN SCINTIGRAPHY (15 uCi)	ANATOMICAL (fair) + FUNCTIONAL (1): rCBF (poor)	INTESTINE	2,200
		STOMACH	1,500
		GONADS	350
		WHOLE BODY	220
		BRAIN	100
^{133}Xe CAROTID INJECTION (5 uCi)	FUNCTIONAL: rCBF (fair)	BRAIN	20
		LUNGS	20
		WHOLE BODY	20
		GONADS	1
^{133}Xe INHALATION (1 mCi/1 x 5')	FUNCTIONAL: rCBF (poor)	BRAIN	100
		LUNGS	100
		WHOLE BODY	100
		GONADS	5
^{81m}Kr CAROTID INFUSION	FUNCTIONAL: rCBF (high) + ANATOMICAL: vessels (poor)	BRAIN	4
		WHOLE BODY	1.5
		HEART (2)	4
		KIDNEYS(2)	4
		GONADS	minimal
		LUNGS	minimal

(1) dynamic study.

(2) due to ^{81}Rb break-through.

To this end, carotid infusion of Krypton-81m has been used in association with a new experimental prototype for emission computerized tomography now available in Italy. This consists of a large field gamma camera which can rotate around the long axis of the patient.

Acquired data are fed into a digital computer and then reconstructed in either transaxial or longitudinal slices.

Patients with established diagnosis of brain disorders were studied. The study showed the feasibility of obtaining both transaxial and longitudinal tomographic reconstruction of cerebral perfusion using ^{81m}Kr and a rotating gamma camera.

Tomographic reconstructions showed the following advantages over conventional views in two dimensions: a) visualization of blood flow distribution within brain structures (gray and white matter, basal ganglia); b) more accurate localization and evaluation of perfusion alterations; c) better definition and evaluation of patterns of collateral circulation; d) greater sensitivity and specificity in detecting and describing blood flow changes during physiological activation studies.

One of the most important applications of this technique is the assesement of the patients with cerebro-vascular disorders, in particular carotid artery occlusion. Comparison of isotope perfusion tomography and conventional x-ray contrast angiography indicated that the former technique provides more accurate and specific information on both indications and effectiveness of external-internal carotid by-pass surgery procedures. This finding is highly relevant to the general issue of the present contract as the isotope procedure is associated with a radiation dose to the patient which is by several orders of magnitude smaller than the dose associated with conventional contrast x-ray procedures such as carotid angiography.

living habit. However, carotid angiography merely provides anatomical information, whereas methods for assessing regional cerebral blood flow (RCBF) have been shown to be more specific and to provide unique information in TIA as well as in other cerebro-vascular disease.

Sensitivity and specificity of carotid angiography (radiation dose to the eye 10,000 millirads) and of ^{81m}Kr brain perfusion studies (radiation dose to the brain 4 millirads) have been assessed for various disease states.

In patients with transient ischaemic attacks, perfusion is similar to normal subjects and angiography is normal. In stroke cases, the tracer distribution usually varies according to the angiographic pattern, but an uneven perfusion is found in 25% of the cases with normal angiography. Patients with occlusion of an intracranial branch of the internal carotid artery show single or multiple areas of impaired perfusion that clearly define the ischemic territory, whose size varies according to collateral flow. To this respect, definition of the perfusion study is superior to that of angiography. In acute vascular lesions, hyperventilation induces collateral filling of the ischaemic area; CO_2 inhalation induces a 'steal' phenomenon indicating that 'vasodilators' may have a negative effect on flow in ischaemic areas. Patients with cerebral tumours may have areas of increased perfusion in the neoformation which are shown better by the perfusion study that by angiography. These patients also show the 'counter steal' phenomenon, that is, an enhanced flow in the pathological vessels (tissue) induced by hyperventilation.

Whereas carotid angiography essentially provides anatomical information, the information provided by isotopic blood flow techniques is mainly functional. Due to the much lower absorbed radiation dose, it would be desirable to substitute blood flow imaging techniques with isotopes for carotid angiography. No doubt these techniques provide the best functional information, but their anatomical information on cerebral vessels is, at the present stage of technology, not yet adequate.

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LENZI G.L., FIESCHI C., FAZIO F.: Regional cerebral blood flow and regional cerebral oxygen utilization in acute cerebral ischemia (abstract). Investigations of brain function, Brice, May 1980 (in press).

Vertragspartner der Kommission:

Universität Erlangen-Nürnberg
Institut für Radiologie

Nummer des Vertrages: 216 - 76 - 7 BIO D

Leiter der Forschungsgruppe: Professor Dr.H.Pauly

Allgemeines Thema des Vertrages:

Organ- und Gewebedosen bei röntgendiagnostischen Untersuchungen:
Ein Beitrag zur mittleren somatischen Strahlenexposition der Bevölkerung
in der Bundesrepublik Deutschland

The research project "Organ and Tissue Doses in Diagnostic Radiology: A Contribution to the Somatic Radiation Exposure of the Population of the Federal Republic of Germany" was initiated in November 1976 and consisted of three parts:

Part I: Measurements of the organ and tissue doses and the energy imparted to the body (integral dose) for the most frequently performed X-ray diagnostic examinations;

Part II: Statistical investigations into the number of the X-ray diagnostic examinations in the Federal Republic of Germany ;

Part III: A contribution to the development and the clarification of the concept: "Somatically Significant Dose".

Ergebnisse des Projektes Nr.1

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Prof.Dr.H.Pauly
Dr.Th.Schmidt
Prof.Dr.G.Hasl

Titel des Projektes: siehe allgemeines Thema des Vertrages.

1: Dose distribution and absorbed energy in the body resulting from X-ray diagnostic examinations (standard and specialized procedures)

1.1 Dose measurements

The dose distributions and the energy absorbed were ascertained on an Alderson phantom. This phantom corresponds largely to the standard human body both with regards to its properties of absorption and dimensions. For the dose measurement small lithium fluoride dosemeters (TLD) were developed which were fittet at a number of sites in this phantom. Under this research project the properties of the dosemeters were studied in detail and their accuracy was ascertained. The distribution of the dosemeters provided a three-dimensional network of measuring points in the phantom. This dose matrix represented the basis for all calculations of dose and energy (3).

1.2 Absorbed energy

The absorbed energy in the total body was determined for a number of X-ray diagnostic examinations. Some of the radiographic examinations together with the absorbed energy are entered in Table 1.1 .

The calculation of the absorbed energy from the measured values is necessarily based on certain assumptions, therefore the absorbed energy is subject to an inaccuracy of roughly ± 30 %.

Table 1.1

Skull	6 to 7.5 mJ
Teeth	0.2 to 0.4 mJ
Lung	2.5 to 3.5 mJ
Stomach	up to 4 mJ
Pelvis (survey view)	up to 25 mJ
Forearm	0.5 to 1 mJ

1.3 Comparison with computations

The values derived from measurements can be compared with calculated values only to a limited extent because the conditions under which radiography is carried out coincide only in some isolated cases. A good agreement was found to exist with the calculations by Kramer/Drexler (2) and Hinz/Kramer/Platz (1) in pulmonary and pelvic radiography.

1.4 Inclusions of fluoroscopy

With the aid of the area-dose product, the radiation exposure in fluoroscopy during a period of one minute was reduced to an equivalent number of radiographic exposures. The equivalent values (F) for fluoroscopy of the stomach, the intestine and the gall bladder were determined (see Table 1.2).

Table 1.2

Organ	F(equivalent number of radiographic exposures to a one-minute fluoroscopy (BVF)	Reference film format
Colon	2.5	24 x 30 cm^2
Gall-bladder	7	9 x 12 cm^2
Gastro-intestinal tract	1.5	24 x 30 cm^2

1.5 Specialized X-ray examinations

The dose distributions and the absorbed energy were also determined for a number of specialized studies (computed tomography, heart catheterization) (5,6,7). Some of the dose distributions obtained with this type of examination deviate to a considerable extent from the distribution patterns obtained in conventional diagnostic radiology. It was found, however, that these specialized studies do not tend to contribute significantly to the total dose burden of the population, however, severe the dose received by the individual patients during such examinations may be.

2: Statistical analyses

Under the research project no separate statistical analyses were carried out with respect to the number of X-ray diagnostic examinations to which the population of the Federal Republic of Germany ist subjected. Therefore, the results gained by inquiries of the German Federal Board of Health (BGA) were resorted to. Based on these statistical values and the absorbed energy for the various X-ray diagnostic examinations, the mean absorbed energy per person and year can be determined for the Federal Republic of Germany. However, this approach is based on the essential assumption that all comparable radiographic examinations were made under more or less identical conditions (voltage, filter, focus-skin distance, film material etc.). This gives the values ascertained a considerable degree of inaccuracy. The mean absorbed energy per inhabitant resulting from X-ray diagnostic work will thus amount to roughly

30 mJ per year.

The contributions made by the individual radiographic examinations to the total absorbed energy are compiled in Table 2.1. The order of the contributions made by the individual examinations to the total absorbed energy is the same as with the genetically significant dose. The contributions resulting from examinations of body areas outside

the abdomen are, however, not so negligible as is the case with the genetically significant dose.

Table 2.1

Regions examined	Percentage of the absorbed energy
Skull	5 %
Teeth	1 %
Chest	5 %
Gastro-intestinal tract	30 %
Pelvis and spine	55 %
Extremities	2 %
Others	2 %

3: Stochastic late effects after partial body irradiation in diagnostic radiology and somatically significant dose

As the ICRP 26 was not published until some time after initiation of the research project, the original objectives underwent changes. Under the research project an attempt was made to estimate from the absorbed energy the risk of malignant neoplasms likely to result from homogeneous partial-body irradiation in diagnostic radiology. This necessitated the assumption that the probability p of neoplasms developing in response to radiation depends on the absorbed energy in the soft tissue of the trunk and the skull and on a mean integral incidence function G_t. This mean integral incidence function G_t has been computed under different conditions from the publication of ICRP 26 to give a value of 0.3 kJ^{-1}. This results in a formula $p = G_t \cdot E_S$.

The question as to what extent this "global method" is equivalent to the calculation from the organ doses according to ICRP 26 needs further investigations. Details can be taken from the paper (4).

Further considerations concern the derivation of a mathematical expression for the so-called "somatically significant dose" or more precisely, the "malignoma-significant dose". This dose parameter was determined for each type of X-ray diagnostic examination, in analogy to the genetically significant dose. Both for the age distribution of the population and for the age distribution of the X-ray diagnostic studies as well as for the incidence of the radiation-induced tumours, analytical functions were used as approximations. After summation of the contributions made by the individual types of X-ray examination, an approximate expression for the "malignoma-significant dose" or the "malignoma-significant absorbed energy" was eventually obtained. Based on plausible assumptions and on the previously mentioned value of 30 mJ per year for the mean absorbed energy per inhabitant, a value of roughly 40 mrem per year is obtained for the malignoma-significant dose for the population living in the Federal Republic of Germany. But this value, too, still contains a considerable margin of uncertainty.

Publication 1:

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Röntgenberichte 7, 170-183 (1978)
(Vortrag: Bayerischer Röntgenkongreß, Bamberg, 18./19.9.1976)

Publication 2:

KRAMER,R., DREXLER,G.:

Zum Verhältnis von Oberflächen- zu Körperdosis in der Röntgendiagnostik.
Vortrag: 7. Wissenschaftliche Tagung der Deutschen Gesellschaft für Medizinische Physik, Heidelberg 1976.
Heidelberg: Dr.A.Hüthig Verlag 1976

Publication 3:

PAULY,H., SCHMIDT,TH., HASL,G.:

Organ doses and integral doses in X-ray diagnosis of the chest and of the head.
IVth International Congress of the International Radiation Protection Association (IRPA), Paris, April 24-30, 1977.
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Publication 4:

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Publication 5:

STIEVE,F.E., SCHMIDT,TH., PIETZSCH,W.:

Strahlenexposition durch die Computertomographie.
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Publication 6:

STIEVE,F.E., SCHMIDT,TH.:

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Röntgenpraxis, 1981 (im Druck)

Publication 7:

VRANA,E., SCHMIDT,TH.:

Strahlenbelastung des Patienten bei Computer-Tomographie.
In: Strahlenschutz in Forschung und Praxis, Band XX (O. Messerschmidt, F. Olbert, Herausgeber). Stuttgart: Georg Thieme Verlag 1980

Contractor:	Gesellschaft für Strahlen- und Umweltforschung mbH - Institut für Strahlenschutz, Ingolstädter Landstr.1, 8042 Neuherberg/Germany
Contract no.:	244 - 77 - 1 BIO D
Head of research team:	Dr. G. Drexler
General subject of contract:	Spectral and physical factors in X-ray diagnosis (in collaboration with contract 245 - 77 - 1 BIO UK, AERE Harwell)

Result of project no. 1

Head of project and scientific staff: Dr. G. Drexler
Dipl.-Phys. W. Panzer
Dr.W.W.Seelentag (till 15.5.79)
Dr.R.Kramer (from 16.5.79 on)

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1. Spectra and physical factors

Improvement of facilities and methods for the determination of the spectral distribution of radiation emitted by X-ray tubes.

1.1 In order to overcome the difficulties (pile up effect) caused by the high output diagnostic X-ray tubes under routine condition an experimental set up was established, consisting of collimators with thin holes, mechanical and optical equipment as well as evacuable tubes with berylium windows to avoid air absorption at large distances.

1.2 To convert the measured pulse height distribution into photon spectra a correction program involving a stripping procedure was developed /1/. This stripping procedure concerns the escape of characteristic germanium-K-radiation and compton scattered photons out of the detector and inefficient photon absorption. The program is based on theoretical Monte Carlo calculations on the detector efficiency and on experimental measurements made with a calibrated Europium-152 source and a monochromatic X-ray source to

evaluate the K-escape fraction. The stripping procedure was implemented on a Wang desk type computer.

1.3 Spectra of radiations were determined, which were emitted by X-ray tubes with molybdenum anodes used for Mammography /2/ and tubes for Dental Radiography /3/, for all possible settings of tube voltage and filtration which are used in routine work or which are proposed to reduce the dose to the patient more efficiently. Spectra were also measured behind the object, to assess how well the spectra were matched to the spectral sensitivity of the image detectors.

For many commercially available films and film screen systems spectral sensitivity was determined in terms of exposure to achieve a certain film density, using a monochromatic X-ray source. Since the exposurerate of this tube was by far lower than under routine conditions, investigations on the reciprocity law failure were carried out /4/.

Concerning Mammography, the most striking finding was that, especially with thick objects, the imaging occurs predominantly by bremsstrahlung, the amount of which actually was suppressed by molybdenum filters directly in front of the X-ray tube. This effect is very contrary to all efforts to reduce patient dose and the use of tungsten tubes in this case (thick objects) should be strongly recommended, because the maximum of emission happens in an energy range where the penetrability of the X-rays is higher. In addition, to profit from the activities mentioned under 1.1 and 1.2 spectra and characteristic data of radiations were evaluated as used in the calibration of therapy and radiation protection dosemeters /5, 6, 7/ superficial therapy and radiation biology /8/.

2. Evaluation of patient dose

2.1 Experimental measurements

Mammography:
Using MIX D as tissue equivalent phantom material entrance doses were measured /2/. Object thickness, tube

voltage, filters and image detectors were varied as under routine conditions. The results show a very strong dependence of dose values on the physical parameters, caused by the severe changes of cross sections in this energy range. The comparatively high dose values can be explained partially by the fact, that the main proportion of radiation, namely the K-radiation from molybdenum, is emitted at energies at which the sensitivity of the image detectors drops drastically.

Dental radiography:
With a simplified skull phantom and thermo luminescence dosemeters the integral doses to the patient were measured under variation of tube voltage and filtration /3/. The dosimetric measurements revealed that the dose reduction using special filters is not significant and efficient reduction can more easily be achieved by an additional aluminum filter than by Samarium filters as proposed in literature.

2.2 Calculation of organ doses

In order to determine organ doses in the human body from X-ray examinations, a suitable computer program has been developed. This program consists basically of a very efficient Monte-Carlo (MC) method which is applied to the heterogeneous MIRD-5 phantom /9/.

The MC-programs RADTEC, ADAM and EVA:
RADTEC was the first version of the MC-program for organ dose calculation /9/. The phantom has the shape and size of the male Reference Man, but includes also female breasts, uterus and ovaries. As the dose to the red bone-marrow is considered very relevant in radiation protection, special efforts have been undertaken to calculate this body dose more accurately. Apart from the old method of calculating the bone marrow dose as the absorbed dose to a homogeneous bone/marrow-mixture weighted with the relative mass fraction of marrow, two additional correctionsfactors are now applied: The ratio of the mass-energy absorption coeffi-

cients of marrow and the homogeneous mixture and a so-called "Spiers-factor" which takes into account the enhancement of dose to bone marrow in trabecular cavities due to the release of photoelectrons in mineral bone for photon energies below 200 KeV.
With regard to radiation quantities relevant in medical radiation protection, the program RADTEC calculates average dose to a body organ normalized to free-in-air patient entrance dose and patient surface dose. The concept of effective and somatic dose equivalent led to the decision to separate the hermaphrodite phantom in a proper male and female version. This resulted in two new sex-specific MC-programs ADAM and EVA, which calculate sex-specific organ doses, especially sex-specific effective and somatic dose equivalent.

Results of organ dose calculation:
Most of the up to now published results have been calculated with the MC-program RADTEC. These are organ dose conversion factors for selected common X-ray examinations /9, 10, 11/. Meanwhile extended calculations have been performed for all relevant X-ray examinations. These data will be published as a handbook to be used in practical situation /13/. The male and female phantom ADAM and EVA, resp., have been incorporated in the MC-code and have passed preliminary test. It will be left to future research work in that field to study sex-specific aspects of body doses and associated radiation risks.

Publications:

/1/ W.W.Seelentag, W.Panzer: Stripping of X-ray bremsstrahlung spectra up to 300 kVp on a desk type computer. Phys. Med. Biol. Vol. 24 No 4, 767-780, 1979

/2/ W.Panzer, G.Drexler, L.Widenmann, L.Platz: Spektren und Dosiswerte in der Mammographie. GSF-Bericht S 518, 1978

/3/ D.F.Regulla, W.Panzer, F.Santner: Filter effects in dental radiography. 5th Intern. Congress of Dento-Maxillo-Facial Radiology, Portland/Oregon, June 28 - July 2, 1980

/4/ W.W.Seelentag, W.Panzer: Reciprocity law failure with film/screen combinations for mammography. Brit.Journal of Radiol. 51, 529-531, 1978

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/7/ W.W.Seelentag, W.Panzer, G.Drexler: A series of X-ray bremsstrahlung spectra with high exposure rates for the calibration of therapy and protection level dosemeters. V. Intern. Conference on Med. Phys., Jerusalem, Aug. 19-24, 1979

/8/ W.W.Seelentag, W.Panzer: Lightly filtered X-ray bremsstrahlung spectra generated at 200 to 300 kV. Rad. Res. Vol. 80 Nr. 3, 409-422, 1979

/9/ R.Kramer: Ermittlung von Konversionsfaktoren zwischen Körperdosen und relevanten Strahlungskenngrößen bei externer Röntgen- und Gamma-Bestrahlung. GSF-Bericht S 556, 1979

/10/ G.Hinz, R.Kramer, L.Platz: Aktuelle Fragen des Strahlenschutzes bei Untersuchungen im Beckenbereich. Röntgen-Berichte 7, 170-183, 1978

/11/ R.Kramer, R.Veit, G.Drexler: Körperdosis-Konversionsfaktoren für computertomographische Untersuchungen. Strahlenschutz in Forschung und Praxis Band XX, 177-189, 1980

/12/ R.Kramer, R.Veit, G.Drexler: Body dose conversion factors for computerized tomography. Intern. Symposium on Biomedical dosimetry, IAEA, Paris, October 27-31, 1980

/13/ R.Kramer: GSF-Report (to be published)

Contractor: United Kingdom Atomic Energy Authority
Atomic Energy Research Establishment
Harwell, Oxfordshire, OX11 ORA, U.K.

Contract No: 245-77-1 BIO UK
Head of research team(s) Dr D H Peirson

General subject of contract: SPECTRA AND PHYSICAL FACTORS IN X-RAY DIAGNOSIS

Title of project nr 2: Spectra and Physical Factors in X-ray Diagnosis
Head of Project and scientific staff: M Marshall, L H J Peaple, R Birch, J A B Gibson, G M Ardran, H E Crooks

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1. Introduction

The aim of this project is to obtain data on the various steps involved in the production of a radiographic image with a view to optimising the quality of the radiograph and reducing the dose to the patient. The objectives have been to study the effect of the relevant parameters on spectrum shape both experimentally and theoretically, to obtain the response of intensifying screens as a function of photon energy, to determine the attenuation by the patient and to study the effect of scattered radiation.

Prior to 1977 techniques had been developed, using NaI and Ge(Li) detectors, for the measurements of X-ray spectra used for the calibration of standard dosimeters and for diagnostic radiology.

Work in the period 1977-80 is presented below.

2. Measurements of Spectra

This work has concentrated on determining the effect of various factors on the spectra and output of diagnostic X-ray machines. The effects of tube voltage (at constant potential), filtration and target angle on spectrum shape and the spectra produced by off-focus radiation have all been determined using the spectrum measuring and unfolding techniques previously developed.

Techniques previously developed for spectral measurements at selected parts of the mains waveform are unsuitable at high currents due to dead-time effects and because diagnostic X-ray generators cannot operate for long enough at high currents for the accumulation of sufficient data. The use of a multi-scaler and single-channel analyser, to measure the variation with time in photon output for different parts of the spectrum, enable the voltage and current ripple to be determined from the knowledge of spectrum shapes. Data can be accumulated rapidly since valid dead time corrections can be made. Hence spectra at any part of the mains voltage waveform can be computed from the spectra and the mean spectrum over any time of interest can be obtained.

3. Theoretical Spectra

A semi-empirical theory to determine X-ray spectra has been devised. Spectra from X-ray generators with tungsten or molybdenum targets can be computed for any voltage in the range 30-150 kV, for any target angle and for any filtration. The theory includes the stopping power and depth distribution of electrons in the target, the differential cross-section for the production of photons and absorption in the target. The theoretical spectra have been compared with measured spectra for a wide variety of conditions and the theory adjusted slightly to give a good agreement. This was necessary probably because the theory does not include relativistic effects.

The calculated exposure rates are consistently high, by (32±5)%, than those measured for a wide range of spectra by ourselves and others. Since losses are expected in practical systems, the agreement is considered satisfactory. Calculated half-value layers agree with measurements by other workers.

4. Response of Intensifying Screens

The variation in narrow beam attenuation with energy has been measured for a wide range of intensifying screens. Hence the composition and effective thickness of the active layer was determined. The proportion of the energy of the incident photons which is absorbed locally and thus can contribute to a useful image was then obtained.

5. Attenuation of Tissue and Tissue Substitutes

The attenuation coefficients of a wide range of tissues and tissue substitutes have been experimentally determined using fluorescent X-rays in the range 10-60 keV.

6. Catalogue of X-ray Spectra

Using the theory described above a wide range of spectra considered useful in diagnostic radiology have been calculated. Relevant parameters such as mean energy, half-value layers, exposure rates and values obtained from a theoretical penetrameter are calculated in each case. Spectra attenuated by various thicknesses of tissue and bone are given. This data, together with the computed response of intensifying screens and various relevant tables has been published by the Hospital Physicists' Association.

7. K-edge Filtration in Mammography

The use of K-edge filters with tungsten targets for mammography has been investigated theoretically. The prediction is that only a small advantage can be obtained compared with the use of aluminium filters.

Experimental checks have not yet been possible due to long delays in obtaining a suitable X-ray tube with low inherent filtration attached to a stable and accurate voltage generator.

8. Scatter Spectra

Preliminary measurements of scatter spectra and their angular distribution have been made but this work was held up due to the failure of the Ge(Li) detector.

9. Measurement of Tube Voltage and Inherent Filtration

Current designs of penetrameter can be used to measure inherent filtration but they are very sensitive to the tube voltage. A penetrameter has been designed specifically to measure inherent filtration which is much less sensitive to voltage but it is still not as precise as is required (estimated precision 0.5 mm). Higher precision is unlikely to be achieved with a penetrameter based on the exposure of film.

An electronic 'instant reading' penetrameter for measuring tube voltage, voltage ripple and inherent filtration has been designed. A prototype has been built using photodiodes but it is neither sufficiently stable nor sensitive enough for operational use. An operational version, using photomultiplier tubes or PIN diodes, would be very expensive.

10. Collaboration with GSF, Neuherberg

This project proceeded in collaboration with the group of G Drexler, GSF, Neuherberg. The programme of work at each laboratory was jointly agreed. Reciprocal visits have been made and there has been a considerable exchange of information. In particular Mr R Birch has visited GSF to install the computer program developed at Harwell on the GSF computer. The program which will calculate diagnostic X-ray spectra is used mainly to provide input data to a Monte-Carlo program to calculate organ doses from diagnostic procedures.

11. Conclusions

A theoretical method of computing the spectra and output of diagnostic X-ray machines has been devised. The attenuation of tissue substitutes and intensifying screens have been experimentally determined and the response of screens calculated. Spectra and other relevant data have been calculated and published. K-edge filtration has no significant advantages in mammography.

The objectives of this project have mainly been achieved. This work will put the physics of diagnostic radiology on a much firmer footing thus making possible the optimisation of the dose to the patient and the radiographic image. Further work requires a knowledge of the scatter spectra produced under various conditions. Unfortunately it has not yet been possible to obtain this due

to instrument failure.

The development of an electronic instrument for quality control in the field has been shown to be feasible but would be very expensive and require further work. Measurement of inherent filtration with a penetrameter to the precision required is unlikely to be possible.

Publications

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III. 6.

ABSCHÄTZUNG DES STRAHLENRISIKOS

EVALUATION OF RADIATION RISKS

EVALUATION DES RISQUES D'IRRADIATION

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Tätigkeitsbericht beschrieben :

Further research work on these subjects will also be described in the following progress reports :

D'autres travaux sur ce thème de recherche sont également décrits dans les rapports suivants :

218-BIA D	GSF, Neuherberg (Gössner)
100-PST D	DKFZ, Heidelberg (Scheer)
101-PST I	ENEL, Roma (Farulla)

Contractant de la Commission : COMMISSARIAT A L'ENERGIE ATOMIQUE, FONTENAY-AUX-ROSES (France)

N° du contrat : 099-76-I-PSAF

Chef du Groupe de Recherche : G. UZZAN

Thème général du contrat : METHODES D'EVALUATION DES CONSEQUENCES DE L'IRRADIATION DES POPULATIONS ET DE L'ENVIRONNEMENT

Les études qui sont menées dans cette action sont réalisées dans le cadre du contrat d'Association EURATOM/CEA N° 099-76-I-PSAF, conclu pour cinq ans à compter du 1.1.1976.

Le programme de recherche s'appuie sur trois projets :

1. Méthodes d'évaluation des doses individuelles et collectives résultant des rejets normaux et des émissions accidentelles

2. Méthodes d'évaluation du détriment radiologique

3. Méthodes d'évaluation des conséquences économiques et sociales de l'irradiation.

Titre du PROJET N° 1 : METHODES D'EVALUATION DES DOSES INDIVIDUELLES ET COLLECTIVES RESULTANT DES REJETS NORMAUX ET DES EMISSIONS ACCIDENTELLES

Chef du projet et collaborateurs scientifiques : A. GARNIER
L. ANGELETTI
A. SAUVE

Ce projet s'intègre dans un programme de recherche ayant pour objectif la mise au point des méthodologies permettant l'évaluation des conséquences de l'irradiation des populations en vue de l'application des recommandations de la CIPR, notamment en ce qui concerne l'optimisation de la protection.

Il comprend des études ayant pour but de contribuer à l'évaluation des doses individuelles et collectives consécutives au fonctionnement des installations nucléaires, ainsi qu'à la représentation de leur répartition à l'échelle européenne en fonction de l'emplacement des sources.

L'estimation des doses collectives exige la prise en compte des transports à longue distance des polluants et des vecteurs de contamination atteignant directement ou indirectement les populations. Ceci explique l'importance prise dans notre programme par l'acquisition de données statistiques (météorologiques, démographiques, économiques) et par le développement de modèles nouveaux (diffusion à grande échelle dans le milieu physique, échanges économiques) destinés à compléter de manière réaliste les modèles classiques.de transferts utilisés pour l'évaluation des doses individuelles. Modèles et données sont actualisés et adaptés autant que possible aux conditions européennes et l'on recourt, le cas échéant, à des études expérimentales.

1. La plupart des données scientifiques et techniques permettant le calcul des doses individuelles à partir de rejets de nature et de composition connues peuvent être empruntées à la littérature ou aux précédents résultats du groupe de recherche. Dans ce domaine, on s'est donc attaché à quelques points précis :

- Inventaire et choix des paramètres et facteurs de conversion des activités en doses, pour les iodes et les gaz rares, qui entrent dans le document de travail, élaboré par la Commission, sur l'étude comparative des facteurs de dose utilisés par les experts ou les autorités des Etats membres pour l'application de l'Article 37. Des valeurs différentes ont été proposées selon qu'il s'agit de rejets de routine ou de rejets accidentels.

- Inventaire et discussion des paramètres de transfert des transuraniens : Np, Pu, Am, Cm. Dans un rapport où sont passés en revue les propriétés nucléaires et physico-chimiques de ces éléments, ainsi que leur comportement dans le sol et les milieux aqueux et leur transfert

dans les végétaux, on présente des tableaux synoptiques rassemblant les valeurs suivantes : coefficients de partage et de diffusion dans les sols et les sédiments, facteurs de transfert en milieu aquatique (eaux douces, eaux de mer, sable, biomasse), facteurs de transfert du sol aux végétaux. Compte tenu du rôle important des formes physico-chimiques dans la chaîne des transferts, on procède à une étude expérimentale de la stabilité de certains composés du plutonium dans l'eau de mer. Les essais effectués d'abord en eau de mer reconstituée mettent en évidence le rôle très important du pH (Doc. 1).

- Etudes complémentaires concernant la vitesse et la rétention des dépôts d'iode sur les végétaux. Une nouvelle analyse statistique de résultats expérimentaux a fait ressortir la nature de la distribution de la concentration de l'iode sur l'herbe, et l'importance de certains paramètres biologiques (teneur en eau de l'herbe) ou des conditions ambiantes (température, humidité, vitesse du vent). Une série d'expériences de laboratoire a montré l'influence de l'humidité atmosphérique sur la captation de l'iode élémentaire par les végétaux (Doc.2).

2. Diverses données statistiques ont été acquises et introduites dans des modèles mis au point pour leur représentation et pour leur utilisation, en collaboration avec diverses équipes européennes.

- Les informations météorologiques, dont il sera question plus loin à propos des modèles atmosphériques, étant fournies dans un système de grille, un mode de représentation analogue s'imposait pour les autres séries de données. Une grille européenne a été conçue et définie pour enregistrer, adapter et utiliser les diverses données.

- Les informations démographiques rassemblées pour les Neuf et pour la Suisse ont été classées en fonction des coordonnées géographiques des localités et représentées dans cette grille unique. Elles entrent dans une banque de données européennes accessible à différents utilisateurs.

- Les informations sur les productions agricoles élaborées dans le même système pour l'étude CEA-NRPB d'impact global sur l'environnement des rejets d'effluents radioactifs en fonctionnement normal dont les résultats rentrant dans la même banque de données, ont été mises à notre disposition.

- Ces trois séries de données sont donc représentées dans une grille à mailles plus ou moins fines, selon leur nature.Disponibles sur bande informatique, elles se prêtent au calcul des concentrations ambiantes, de la répartition des doses d'inhalation et d'irradiation externe, et de la répartition des sources de contamination par voie d'ingestion.

- Par contre, le recueil des informations concernant les opérations de collecte, transformation, échanges et distribution des produits destinés à l'alimentation n'a été possible qu'au niveau des régions et parfois même des Etats, en raison de la complexité des circuits et souvent aussi de l'insuffisance des données. Des modèles ont été spécialement mis au point pour chaque groupe de produits alimentaires importants, chacun posant des problèmes particuliers. On a étudié successivement les céréales, les produits de la mer, les produits laitiers et en dernier lieu, les viandes ainsi que les produits destinés à l'alimentation animale, et les cultures irrigables (Doc. 3).

Ces modèles permettent, dans l'hypothèse de contaminations plus ou moins élevées au stade de la production (par exemple en cas d'accident) de tester l'influence de l'ensemble des opérations intervenant entre la production et la consommation sur la répartition des contaminations globales et individuelles moyennes dans les régions consommatrices. On s'est attaché à mettre en évidence les régions d'influence et les régions sensibles, ce qui peut donner des indications utiles pour adapter des programmes de surveillance à des circonstances particulières.

3. L'évaluation de la contamination des milieux récepteurs à l'échelle européenne nécessite des modèles de diffusion adaptés aux distances de plusieurs centaines de kilomètres. La localisation des contaminations éventuelles impose le recours à des modèles de trajectoires, permettant de prévoir la probabilité d'occurence ou la probabilité d'atteinte selon qu'il s'agit de rejets de routine ou de rejets accidentels.

- Ce problème a fait l'objet d'une étude approfondie dans le cas des effluents gazeux, dont rend compte un rapport détaillé en cours de rédaction. La mise au point du modèle MESOS apparaît comme un résultat essentiel, tant pratique que théorique. La nécessité de disposer de données très complètes pour mettre en oeuvre ce modèle perfectionné a conduit à étendre, améliorer et adapter aux programmes de calculs les informations recueillies à l'échelle européenne. On a également rassemblé des données locales destinées à compléter les précédentes ou à vérifier des estimations. Ces acquisitions réalisées au prix d'efforts importants soutenus par la Commission des Communautés contribuent à la connaissance du milieu ambiant européen et à une harmonisation des méthodes (Doc. 4).

D'autre part, des analyses de sensibilité et des comparaisons avec d'autres modèles ou avec des résultats observés (réalisées entre autres dans le cadre des activités du groupe d'experts météorologique pour l'application de l'Article 37) ont permis de mettre en évidence et d'évaluer l'influence de divers paramètres sur les résultats des calculs de doses. Cette démarche permet en outre d'étayer des hypothèses simplificatrices et d'en préciser les limites d'application ou la fourchette de validité, en vue du choix des modèles les plus appropriés aux problèmes à résoudre (impact des installations en fonctionnement normal, rejets concertés ou accidentels). Les hypothèses concernant les conditions atmosphériques à la source et surtout leur évolution le long de la trajectoire sont les plus importantes puisqu'elles sont déterminantes pour la localisation des zones contaminées. On procède donc à un examen comparatif de la répartition des concentrations atmosphériques et des doses obtenues sur le territoire européen au cours d'une ou deux années, à partir de plusieurs sites (Mol (1973 et 1976), Heysham et Karlsruhe (1973), Ispra et Cadarache (1976).

- La modélisation de la diffusion dans les eaux continentales et marines pose, à longue distance, des problèmes très délicats. Avant de l'aborder, on a testé l'influence des niveaux de contamination de l'eau des différentes zones de pêche sur l'ingestion de radionucléides par les consommateurs. On peut en conclure que si l'on traite le problème à l'échelle globale, seules des différences de niveau très importantes entre les zones de pêche, auraient un effet sensible sur

la répartition des doses dans les régions de consommation, car ces différences sont modifiées par de multiples facteurs. On a vérifié d'autre part la bonne concordance entre les résultats de l'application à des rejets connus, d'un modèle simple, avec des résultats de mesures. Par conséquent, on peut préconiser d'une part un modèle relativement simple pour les évaluations globales et moyennes ; d'autre part, des modèles particulièrement adaptés à des problèmes précis, et tenant compte des phénomènes physico-chimiques et biologiques et des paramètres propres aux sites aussi bien que des équations classiques de diffusion. Ceci est encore justifié par les interactions possibles entre milieu marin et milieu atmosphérique, importantes pour les voies d'exposition directe.

Ces investigations restent à poursuivre, compte tenu des programmes existant par ailleurs, et de la disponibilité de résultats de mesures ou de résultats expérimentaux.

4. La mise en oeuvre de ces méthodes permet, pour chaque source considérée :

- Dans le cas des effluents gazeux, l'évaluation des taux de contamination atmosphérique et de leur répartition sur le territoire européen, ainsi que celle des doses individuelles et collectives correspondantes. Des exemples de résultats seront présentés dans le modèle de grille européenne qui a été défini.

- Dans le cas des effluents liquides, bien qu'il soit possible, avec les modèles existants (diffusion, transferts, échanges), d'obtenir des évaluations d'impact radiologique global aussi bien que de doses à des groupes particuliers de population, des études sont encore nécessaires pour des estimations prévisionnelles plus détaillées tenant le plus grand compte possible des effets de sites.

Titre du PROJET N° 2 : METHODES D'EVALUATION DU DETRIMENT RADIOLOGIQUE

Chef du projet et collaborateurs scientifiques : Dr L. KARHAUSEN
Dr R. MAXIMILIEN
Dr W. SKUPINSKI

Ce programme de recherche a pour but d'améliorer l'état des connaissances relatives à l'évaluation du détriment radiologique chez l'homme notamment dans la gamme des faibles doses en développant les différentes approches complémentaires suivantes :

. Synthèse des données expérimentales et épidémiologiques de la littérature en se référant, le cas échéant, à toute information utile hors du cadre de la radioprotection (comparaison de risques).

. Etudes épidémiologiques de populations soumises à diverses sources d'irradiation : médicale, professionnelle, voire naturelle.

. Recherche et modélisation des données expérimentales susceptibles de contribuer à l'évaluation de la relation dose-effet dans les secteurs où les observations sur l'homme sont insuffisantes.

I - APPROCHE BIBLIOGRAPHIQUE

Des rapports de synthèse de la littérature ont été entrepris dans un double esprit :

- Un premier type de revue bibliographique se propose d'établir un bilan des connaissances acquises dans un domaine particulier pour dégager les perspectives de futures recherches. A cet égard, les approches conduisant à l'évaluation du risque génétique chez l'homme ont fait l'objet d'une analyse critique (Université de Leiden). Dans une seconde monographie, les données récentes relatives à l'évaluation des effets somatiques des radiations ionisantes ont été réunies en vue d'actualiser le dernier rapport de l'U.N.S.C.E.A.R. et d'orienter le programme des enquêtes épidémiologiques (Institut de Recherches Mario Negri, Milan). Enfin, une série de travaux a été consacrée à l'étude de la relation dose-effet chez l'animal irradié par divers nucléides de la famille des transuraniens ; ces dernières études procèdent à une analyse comparative des effets cancérogènes et non cancérogènes de l'américium, du cérium et du plutonium.

- Un second type de revue bibliographique a pour but d'envisager les possibilités et les modalités de mise en oeuvre d'enquêtes épidémiologiques. Une synthèse des résultats d'études épidémiologiques récentes effectuées sur des individus irradiés dans leur enfance pour teigne du cuir chevelu a été réalisée afin d'examiner l'opportunité d'une enquête plus approfondie. Une seconde revue bibliographique, traitant de la

radiothérapie de l'acné, analyse des orientations à donner à une enquête épidémiologique en cours sur le sujet.

II - APPROCHE EPIDEMIOLOGIQUE

L'étude des populations constitue l'outil préférentiel de l'évaluation du détriment radiologique ; l'approche épidémiologique a donc été développée selon les principaux axes suivants :

1/ Etude de populations exposées aux radiations ionisantes dans le passé. La principale source d'informations disponibles en cette matière, est représentée par l'irradiation pour raison médicale (radiodiagnostic, radiothérapie). Dans la mesure du possible, les enquêtes ont tenté de prendre en compte les interactions éventuelles des radiations ionisantes avec d'autres facteurs dans l'induction des effets sanitaires.

. A la suite des travaux récemment publiés sur l'accroissement du risque de tumeurs cérébrales, parotidiennes et thyroïdiennes consécutives à un traitement radiologique pour dermatose bénigne de la tête et du cou, une étude épidémiologique des conséquences à long terme de la radiothérapie pour acné, sycosis barbae ou encore eczéma, a été lancée en Irlande (Médical School, Dublin). Les dossiers médicaux provenant de huit villes britanniques ont été regroupés et ont permis d'identifier plus de 14 000 patients traités par les rayons X mous dans les années antérieures à 1945. Le devenir de ces sujets a été recherché à l'aide du registre national des décès et des registres national et régionaux du cancer. L'étude actuelle compare les taux de mortalité par cancer chez les groupes de sujets irradiés (répartis en fonction des doses totales reçues) à ceux de la population générale du Royaume-Uni. Une analyse ultérieure d'un échantillon du groupe irradié, comportant notamment un calcul des doses délivrées à différents organes, devrait permettre une évaluation plus précise du risque.

. Une autre étude épidémiologique du même type a pour objet l'interaction possible du tabagisme et du radiodiagnostic dans l'induction des cancers (St George's Hospital, Londres). La population examinée comporte 30 000 sujets exposés à différents protocoles de radiodiagnostic dans les années 1950-1960 dans le cadre de l'évaluation des programmes de dépistage systématique des cancers du poumon. Les données démographiques, médicales et radiologiques ainsi que les habitudes tabagiques de cette population avaient fait l'objet, à l'époque, d'une description soigneuse. Le présent travail consiste à reprendre ces dossiers et à rechercher le devenir des sujets auprès des médecins de famille, des employeurs et du service chargé de la centralisation des certificats de décès. Encore incomplète, cette étude devrait permettre de déceler un effet multiplicatif du tabagisme de 1,6 avec une probabilité de 90%.

2/ Etude de populations professionnellement exposées aux radiations ionisantes. De telles enquêtes supposent une estimation précise des doses reçues par le personnel ainsi que la possibilité de disposer d'effectifs suffisants pour autoriser l'analyse. Il est donc apparu nécessaire d'étudier, dans une étape préliminaire, les modalités de mise en oeuvre d'un registre central des travailleurs de la Communauté Européenne. Une étude de faisabilité a mis en évidence de multiples difficultés d'ordre déontologique et d'ordre statistique tenant respectivement à des problèmes de confidentialité et d'accessibilité aux données (St George's Hospital, Londres).

3/ Dans le même ordre d'idées, l'établissement d'un bilan d'exposition de la population européenne, tenant compte des différentes composantes d'irradiation (médicale, naturelle, ...) a été envisagé et fait l'objet d'études méthodologiques :

. En ce qui concerne l'irradiation naturelle, deux enquêtes sont menées (Trinity College de Dublin) (Ecole de Santé Publique de Rennes) pour chercher à définir des protocoles de mesures adéquats pour évaluer l'irradiation naturelle à l'échelle de la région ainsi que pour tenter d'élaborer un indice représentatif de la dose population correspondante. Pour caractériser l'exposition externe, la distribution étroite des valeurs enregistrées dans chaque unité géographique (commune en France et district électoral en Irlande) suggère la possibilité d'adopter la moyenne des valeurs relevées à condition que la densité de la prospection ait été suffisante. Des compléments d'investigation sont envisagés pour vérifier la validité de procédés d'enregistrement simplifiés ainsi que pour prendre en compte le paramètre géologique dans l'exploitation des résultats. Par ailleurs, ces études soulignent l'importance de l'irradiation domestique (irradiation externe et radon dans les constructions) et la nécessité d'élaborer des protocoles de mesures appropriés. Un début d'analyse des effets sanitaires ne met en évidence aucune variation significative du risque dans des zones où le niveau moyen d'exposition externe est différent.

. En ce qui concerne l'irradiation pour raison médicale et plus particulièrement l'exposition à des examens radiodiagnostiques, diverses options sont envisagées pour effectuer une cartographie européenne de cette composante.

III - DONNEES EXPERIMENTALES

La simulation des conditions d'exposition de l'homme par l'expérimentation animale constitue une approche supplémentaire de l'évaluation du détriment radiologique et trouve une place privilégiée chaque fois que les données humaines sont difficiles à rassembler. Elle pose néanmoins divers problèmes tant sur le plan de la méthodologie d'exploitation des résultats que sur celui de l'extrapolation des données à l'homme.

1/ Sur le plan méthodologique, les résultats d'expériences d'exposition de rats au radon avec ou sans cofacteurs (reproduction de la situation des mineurs d'uranium) ont été analysés. Des difficultés d'interprétation similaires à celles que l'on rencontre en épidémiologie ont été mises en évidence, notamment dans l'estimation de la prévalence réelle du cancer du poumon du fait de la mortalité précoce par affections intercurrentes dans certains lots d'animaux, ou encore dans la détermination de la cause réelle du décès (mort avec cancer et mort par cancer sont en effet souvent difficiles à distinguer). Ces problèmes étant pris en compte, il a été possible de montrer que l'excès de mortalité chez les animaux recevant les doses les plus élevées, n'est pas dû à l'augmentation de la prévalence du cancer du poumon, et que le raccourcissement de l'espérance de vie peut s'expliquer par la présence de lésions non néoplasiques du parenchyme pulmonaire. La prévalence du cancer pulmonaire au bout d'une période donnée écoulée depuis l'exposition croît avec l'âge de l'animal à l'exposition. L'inhalation de fumée de cigarette augmente le risque de façon considérable. L'effet d'autres

cofacteurs (SO_2, Fe 59, Cérium 144) et de divers agents chimiques anticancéreux a été étudié. Les futurs protocoles de recherche sont discutés à la lumière des enseignements de cette analyse (nécessité de randomisation, de dimensionnement de l'effectif de rats étudiés en fonction de la dimension de l'effet attendu, de prise en compte de la dérive génétique de la souche au cours du temps).

Dans un autre domaine, les lésions induites au niveau du rein par les radiations ionisantes ont été comparées chez le rat et chez l'homme (Association Claude Bernard). L'étude de deux lignées de rats met en évidence une susceptibilité différente à l'irradiation tant pour les lésions tumorales que pour les lésions non tumorales. Ces différences sont rapportées à une différence de prédisposition génétique du risque rénal. L'analyse a été poursuivie en vue de vérifier si les lésions observées dans les maladies autoimmunes chez l'homme sont comparables à celles observées chez le rat après contamination par des émetteurs α . L'étude conclut que si l'irradiation entraîne des lésions proches de celles observées au cours du syndrome de Sjögren, l'irradiation d'une immunodépression ou au contraire d'une immunostimulation n'entraîne pas de modification des lésions.

2/ En ce qui concerne l'étude des effets somatiques pathologiques provoqués par les rayonnements ionisants et par certaines autres nuisances physiques ou chimiques, des recherches expérimentales ont été menées dans deux directions principales (SPPS-SRTE du CEA-DPr) : Etude des effets à court terme provoqués par de fortes doses et étude des effets à long terme que l'on observe soit chez les individus ayant survécu à la phase initiale d'une irradiation aigüe, soit chez ceux qui ont été exposés à des doses plus faibles. Dans chacune des deux directions, en plus des études sur les effets pathologiques, des recherches sont consacrées aux problèmes du diagnostic et de la thérapeutique.

Titre du PROJET N° 3 : METHODES D'EVALUATION DES CONSEQUENCES ECONOMIQUES ET SOCIALES DE L'IRRADIATION

Chef du projet : A. OUDIZ

Les travaux relatifs à ce projet ont commencé en août I977. On a commencé par montrer que"l'évaluation des conséquences économiques et sociales de l'irradiation", intitulé originel du projet, fait partie intégrante d'un tout constitué en réalité par l'étude des méthodes permettant d'assurer " l'optimisation " de la radioprotection, conformément aux recommandations 22, puis 26, de la CIPR.

I - LES METHODES D'OPTIMISATION

Il a dès lors été procédé à l'application de diverses méthodes d'aide à la décision dans la perspective de l'optimisation de la radioprotection.

La première méthode abordée a été l'analyse multicritère en I978 pour laquelle on a repris les données de l'Environmental Protection Agency (USA) relatives au 40 CFR I90, l'une des rares études susceptibles à cette date de fournir des informations sur les coûts et les efficacités de divers systèmes de protection du public, dans le cadre du fonctionnement normal du cycle du combustible PWR. L'étude a porté sur une quarantaine de systèmes de traitement des effluents liquides et gazeux relatifs aux diverses installations du cycle du combustible. Elle a permis de sélectionner les " meilleures " de ces options sous le triple point de vue de la réduction de la dose individuelle dans les groupes critiques, de la dose collective et du coût des systèmes. Une telle étude a eu pour but essentiel de mettre en évidence le caractère multidimensionnel des choix de protection de la population en général, des groupes critiques,des coûts, etc..et de montrer que, dès lors, il existe des méthodes alternatives à l'approche coût-avantage, permettant dans certains cas de mieux tenir compte de la multidimensionnalité.

Suite à cette étude, on a mis en oeuvre en I979 une méthode par fonction d'utilité. Un double but a été poursuivi : d'une part, présenter une autre méthode quantitative, suite à l'analyse multicritère Electre I, d'autre part, prendre en considération la question du transfert éventuel de risque du public vers les travailleurs dans le cadre de l'optimisation des niveaux de dose au public. La base de données a consisté cette fois en des données originales françaises recueillies par le Centre d'Etude sur l'Evaluation de la Protection dans le domaine Nucléaire (CEPN, Fontenay-aux-Roses, France) en ce qui concerne les performances et les coûts des systèmes de protection prévus pour les réacteurs de 1300 MWe. La

connaissance de l'influence de ces systèmes sur la dose collective travailleurs reste actuellement très approximative et l'étude s'est dès lors basée sur une évaluation d'experts en ce qui concerne cet aspect particulier.

L'étude a porté sur le choix des systèmes de traitement compte tenu d'une quadruple préoccupation : réduire les doses individuelles dans les groupes critiques, réduire les doses collectives, tenir compte du transfert éventuel d'une partie de la dose au public vers les travailleurs et enfin considérer le coût des systèmes. L'étude tend à montrer que certains systèmes sont susceptibles d'induire une dose collective aux travailleurs supérieure à celle qui est retirée dans le public. Une telle conclusion est cependant dépendante de la validité de l'évaluation d'expert que l'on a dû effectuer. Cependant, les développements actuels de la dosimétrie individuelle et collective dans les centrales nucléaires sont susceptibles de préciser à court terme les hypothèses adoptées provisoirement dans cette étude.

Une autre étude a porté en I979 sur la revue des principales méthodes envisageables dans le domaine de l'optimisation ou plutôt de la rationalisation des choix de protection. Elle a permis de présenter et de comparer sur un même exemple numérique, tiré de l'étude de l'EPA déjà citée, les méthodes coût-efficacité, coût-avantage, multicritère Electre I et fonction d'utilité. Il n'est pas possible de tirer une conclusion générale en terme de supériorité d'une méthode sur les autres, étant donné que l'intérêt relatif de celles-ci dépend de l'objectif décisionnel poursuivi et des caractéristiques du problème traité. Il est probable cependant qu'une méthode aussi complexe qu'Electre ne devra être mise en oeuvre que dans le contexte de décisions " lourdes ", dans lesquelles la multidimensionnalité des critères de choix nécessite une technique conçue pour concilier des points de vue multiples et hétérogènes.

Suite aux travaux précédents, consacrés essentiellement à l'optimisation de la protection du public, le programme a été orienté en I980 vers celle de la protection des travailleurs. Dans ce but, on a procédé à une étude de faisabilité destinée à identifier les secteurs susceptibles de donner lieu ultérieurement à des études décisionnelles. Il apparaît que, parmi les diverses installations du cycle du combustible PWR, ce sont les mines d'uranium, les centrales nucléaires et les usines de retraitement qui justifient, par l'importance des doses individuelles et/ou collectives reçues par le personnel, que l'on envisage en priorité des études d'optimisation. Cependant, le problème de l'accessibilité des données concernant les systèmes de protection et leurs coûts constitue une contrainte importante dont on devra tenir compte à l'avenir.

Une étude consacrée aux mines d'uranium a été effectuée dans ce contexte. Elle présente un modèle théorique en fonction des divers paramètres de la mine, en particulier des aérages primaire et secondaire. Ce type d'approche permet de dégager les principales variables d'action sur lesquelles on pourrait agir pour diminuer l'irradiation externe ou la contamination du personnel.

II - ORGANISATION D'UN SEMINAIRE CONSACRE A L'OPTIMISATION

En octobre I979, l'Association EURATOM/CEA a organisé, en liaison avec les Directions Générales V et XII de la Commission des Communautés Européennes, un Séminaire Scientifique Européen consacré à l'optimisation de la radioprotection.

Il a permis un échange de vues approfondi entre les spécialistes des méthodes d'optimisation et les responsables chargés de la radioprotection dans les divers pays membres.

Les discussions ont fait apparaître que le processus d'optimisation ne saurait être conçu comme un substitut aux pratiques décisionnelles actuelles, mais plutôt comme un complément utile permettant parfois d'orienter des décisions obéissant en général à des déterminations complexes.

Le Séminaire a conduit à dégager une position de l'Association vis-à-vis des travaux effectués dans d'autres pays :

- Il y a intérêt à ne pas fixer le recours à telle ou telle méthode d'optimisation.

- Il y a intérêt à ne pas fixer de façon définitive la valeur de certains paramètres importants, tels que la valeur de l'homme Sievert lesquels peuvent différer selon les circonstances d'un pays à l'autre.

- Il y a intérêt à bien séparer au niveau des études, les approches technico-économiques de la radioprotection et les analyses de l'acceptabilité sociale des risques ou des technologies.

- Le résultat de l'optimisation ne constitue en définitive qu'une donnée parmi d'autres dans le contexte de la prise de décision.

Toutes les communications du Séminaire, ainsi que les discussions, seront publiées durant l'année I98I dans les " Actes du Séminaire ".

III - LES ETUDES COMPARATIVES

La comparaison entre diverses industries permet de fournir des éléments d'appréciation indispensables pour situer le risque radiologique résultant des activités électronucléaires vis-à-vis d'autres risques industriels. Une telle comparaison met en évidence les niveaux de protection dans les divers secteurs, les coûts qu'ils entraînent, les niveaux de risque résiduels ainsi que les coûts implicites de l'effet sanitaire évité dans les secteurs considérés.

La connaissance de ces coûts implicites éclaire le problème de la détermination de la valeur de l'homme-Sievert pour le public et les travailleurs dans l'industrie nucléaire.

La première comparaison effectuée porte sur les centrales thermiques classiques. On a identifié un certain nombre d'actions de prévention des risques liés à la pollution atmosphérique par les oxydes de soufre et les poussières. On a évalué ensuite les coûts de ces actions de prévention et leur efficacité en termes de réduction des émissions d'oxydes de soufre et de poussières. Cette étude a été prolongée par une autre, dans laquelle on a procédé à l'évaluation de l'efficacité en termes d'effets sanitaires évités (mortalité dans des groupes à haut risque, liée à une aggravation d'affections respiratoires et cardiovasculaires). Cette étude donne le coût implicite de l'effet évité grâce au recours au fuel-oil à basse teneur en soufre dans des centrales thermiques situées dans la périphérie de Paris.

La seconde comparaison porte sur le risque professionnel dans les industries utilisant l'amiante (amiante ciment amiante manufacturé).

L'étude comporte trois volets : la nature et l'ampleur des risques associés à l'amiante, les mesures visant à réduire les niveaux d'exposition et leur coût, la synthèse mettant en relation la réduction du risque et les coûts associés.

La littérature internationale concernant les risques liés à l'amiante est très riche et elle a permis de dégager les relations exposition-risque utilisables pour les diverses pathologies (asbestose, cancers du poumon et mésothéliomes).

L'accès aux données concernant les dispositifs de réduction du risque amiante, aux coûts associés, et aux taux d'empoussièrement avant et après recours à ces dispositifs s'est, par contre, avéré très difficile, compte tenu de la réticence du milieu industriel concerné à fournir des données. Le travail a donc été effectué en s'appuyant sur les données publiées dans certains pays tels que l'Angleterre, les Etats-Unis et le Canada.

Document 4

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : BC-1939
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Nuclear Power Section, Mechanical Engineering Department

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ATMOSPHERIC TRANSPORT OF RADIOISOTOPES AND THE ASSESSMENT OF POPULATION DOSES ON A EUROPEAN SCALE

1. Original Aims

An important aim was to develop further the MESOS model, which had been developed under an earlier one year contract. Into the model were to be built the diurnal development of the mixing layer allowing for the nature of the underlying surface and the ability to calculate time integrated atmospheric concentrations, dry deposition and wet deposition. The model was intended to be as realistic as possible, for example including rain when and where it occurred. Data bases were to be developed to provide the necessary meteorological parameters and it was required to include all the Community countries within the area of study. In collaboration with the Association EURATOM-CEA, data on population distributions and data on the production of foodstuffs were to be incorporated for use with annual average contamination predictions. A final objective was to investigate the potential of the model for treating accidental releases, to assess the influence of meteorology on the probability of various consequences and to identify adverse meteorological conditions.

2. Outline of the method

The MESOS model traces the history and development of a sequence of puffs released every 3 hours into the evolving synoptic situation. Material released in between is treated as a continuous sequence of puffs following itermediate trajectories, for which contamination can be found by interpolation. Thus, unlike most other models, the important lateral spreading of material due to divergence of trajectories in the synoptic pressure field is allowed for, making the model a powerful tool for studying accidental releases of various durations.

Realistic trajectories for the tracked puffs are calculated from geostrophic winds moderated and adjusted according to the likely wind profile in the current local stability conditions, and the current vertical distribution of material. A puff is considered as a vertical rectangular column, expanding laterally due to small scale turbulence and wind shear, and evolving vertically according to the changing state of the boundary layer (ie. the lowest 1 or 2 km of the atmosphere). This is treated as a well mixed lower layer - the mixing layer - surmounted by a series of stratified layers of stable air with negligible turbulence. The changing depth of the mixing layer is assessed taking account of wind strength, underlying surface land or sea, cloud cover, and insolation, following a diurnal cycle. Initially, vertical dispersion of material depends on the appropriate sequence of stability categories along the trajectory, until the puff expands to fill the current mixing layer. Thereafter a deeper

mixing layer may dilute material over a greater depth; or a shallower mixing layer may lead to isolation of material above an inversion whence it cannot by depleted by deposition, until re-entrained again into a deeper mixing layer. Depletion by natural decay, dry depositon, and wet deposition according to the intensity of precipitation where and when it occurs are also included.

The model has been applied to five nuclides, Kr-85, Xe-133, Xe-135, Cs-137 and I-131 (the latter having two forms with different deposition parameters). For Kr and Xe only time integrated concentrations are calculated, but for Cs and I separate dry and wet deposition also. MESOS results are interpolated to regular grids over which population distributions and agricultural producation for consumption has been supplied through the collaboration with the Association EURATOM-CEA. Five notional release sites have been considered in different Community countries; Mol, Heysham and Karlsruhe with a 1973 data base and Mol, Cadarache and Ispra with a 1976 data base.

3. Continuous (planned) release results

Maps have been produced showing average air exposure and wet and dry depositon, for hypothetical unit releases from the selected sites. In addition, distributions of total contamination in foodstuffs at the point of production for consumption have been produced, enabling collective dose to be calculated. Results for Mol for 1973 and 1976 do not show substantial differences in these annual average calculations. Simple straight line trajectory models have been devised and compared with the MESOS results for annual averages. Among factors limiting the accuracy of such simple models are, for example, the diversion of trajectories by the Alps, the tendency of westerly trajectories to turn back to the east and the effect of orographic rain.

4. Accidental (unplanned) release results

Recognising that the model was applicable to accident situations, its potential for treating releases of durations of multiples of 3 hours has been explored. An additional benefit has been that the model has met the requirements of the C.E.C. Meteo Experts group concerned with estimating probability distributions of levels of contamination at a point in a neighbouring state in the event of an accident.

By considering results for the five sites statistically, for release periods from 3 hours to 7 days, it has been shown that release duration is an important parameter. Situations leading to high contamination at selected receptor points have been analysed in detail, considering such factors as the synoptic situation, distance and time travelled, maximum vertical dilution attained and wind speed at the receptor point. This has been done for three nuclides of different behaviour. Comparison of results has been made with other models TALD and CAGNETTI; since these models do not reflect fully the variations in meteorological conditions they predict a more restricted range of contamination.

The possibility of fitting simple parametric functions to probability distributions has been explored and seems encouraging. This offers the prospect of organising the very large amount of data and making it available for practical use. It is intended to explore the potential of this approach for various nuclides and the range of source and receptor points.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 002-EIR
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ETUDE DE L'IRRADIATION NATURELLE EN IRLANDE

Cette étude s'inscrit dans le cadre des recherches méthodologiques visant à établir un bilan d'exposition de la population européenne à l'irradiation d'origine naturelle et s'articule à ce titre avec une enquête du même type menée en Bretagne. L'optique générale du travail est de chercher à définir un protocole de mesure approprié pour couvrir l'ensemble du territoire par un réseau de relevés des niveaux d'irradiation naturelle et de tenter d'élaborer un indice représentatif de la dose population par unité géographique.

I - EXPOSITION EXTERNE

Dans un premier temps, l'ensemble du territoire irlandais a été prospecté de façon régulière à l'aide d'une chambre d'ionisation à haute pression, type RSS 111 (264 relevés). Les valeurs enregistrées varient de 35 mR/an à 195 mR/an et la moyenne est de 72 mR/an (y compris le rayonnement cosmique dont la valeur est de 30 mR/an). Les niveaux d'activité les plus faibles sont relevés sur les terrains sédimentaires et les plus élevés correspondent aux roches éruptives et intrusives. Rapportée à la distribution de la population irlandaise, la dose moyenne liée à l'exposition externe est évaluée à 76 mR/an, c'est-à-dire de l'ordre des valeurs décrites en Europe.

Dans un second temps, l'étude d'une zone sélectionnée (Midlands et Sud Est de l'Irlande) a été entreprise afin de décrire de manière plus approfondie l'irradiation externe en fonction de la diversité de l'environnement humain et naturel. Les mesures sont effectuées aussi régulièrement que possible en découpant préalablement les zones de prospection à l'aide de la grille nationale de référence et de diverses cartes géologiques. 2756 relevés ainsi collectés représentent une densité d'enregistrement de l'ordre de 12 mesures/62km2, soit au moins 3 mesures par District Electoral Division (maille la plus fine permettant d'obtenir les informations démographiques précises). Les résultats de cette enquête confirment que les zones de roches éruptives et de granits sont responsables des niveaux d'exposition externe les plus élevés (moyennes respectives: 11,8 et 10,4 µrad/h) et que les terrains calcaires sont beaucoup moins actifs (moyenne relevée : 6,6 µrad/h). La moyenne de l'ensemble des relevés collectés dans la région est de 7,9 µrad/h et l'étendue des relevés est de 5-20 µrad/h.

II - EXPOSITION DOMESTIQUE

- Etant donné la part importante de l'irradiation domestique subie par la population, une campagne de mesure de l'exposition externe et de la concentration de radon a été entreprise sur un lot restreint de constructions :

. En ce qui concerne l'irradiation externe, des mesures ont été effectuées à l'intérieur et à proximité immédiate de 119 habitations. Les mesures intérieures sont réalisées dans trois pièces différentes . La moyenne des valeurs enregistrées à l'intérieur des constructions est de 10 µR/an tandis que celle des valeurs relevées à l'extérieur est de 8,3 µR/an , ce qui met en évidence un surcroît d'irradiation externe à l'intérieur des bâtiments de l'ordre de 25%. Rapportés aux matériaux de construction, les résultats montrent que les niveaux les plus élevés correspondent aux constructions utilisant la pierre de pays et les roches éruptives alors que les niveaux les plus faibles correspondent aux habitations en béton. Toutefois, il existe des fluctuations très importantes du niveau d'irradiation moyen indépendamment du matériau de construction comme le montre l'étude d'habitations identiques situées dans des régions différentes voire même voisines.

. En ce qui concerne la mesure du radon, une enquête de faisabilité a été effectuée dans 10 habitations choisies de façon aléatoire. Des prélèvements d'air successifs ont été réalisés dans les conditions standardisées (pièces closes depuis 12 heures au moins ..). Les valeurs observées varient de 0,1 à 2,5 pCi/l , c'est-à-dire très inférieures à celles relevées dans une étude similaire effectuée en Bretagne. Aucune relation entre la concentration de radon et l'irradiation externe n'a pu être mise en évidence, ce qui suggère que le K^{40} représente la source principale de l'expositon externe. De même, l'importante fluctuation temporelle des valeurs mesurées dans une même construction n'a pu être expliquée par les conditions météorologiques.

III - ETUDE DE LA MORTALITE PAR CANCER

Les niveaux d'irradiation naturelle observés dans la zone sélectionnée pour l'étude approfondie présentent des variations importantes selon les sites. La moyenne des valeurs observées a été calculée pour chaque " District Electoral Division " et ceux-ci sont regroupés dans des classes de niveau d'irradiation externe. L'analyse des données sanitaires ne met en évidence aucune association entre la mortalité par cancer et les niveaux d'irradiation naturelle. Cette étude porte sur 3,5 millions d'années-personnes, ce qui permettrait d'identifier un risque d'induction de cancer supérieur à celui calculé par l'extrapolation linéaire du risque des forte aux faibles doses d'irradiation.

CONCLUSION

Cette étude fournit des données originales sur l'irradiation naturelle en Irlande que l'on pourra secondairement intégrer dans un bilan de l'exposition de la population européenne aux radiations ionisantes. Elle souligne par ailleurs la nécessité d'approfondir l'étude de l'exposition domestique (irradiation externe et radon dans les constructions) principale source de l'irradiation de la population.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 008 F
Service de Radiopathologie et de Toxicologie Expérimentales
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RECHERCHE EXPERIMENTALE SUR DES CANCERS RADIO-INDUITS
(ETUDE DES MECANISMES, ANALYSE MATHEMATIQUE, ETUDE DES EFFETS COMBINES)

Les recherches sont menées dans deux directions principales :

- les effets à court terme provoqués par de fortes doses,
- les effets à long terme que l'on observe soit chez les individus ayant survécu à la phase initiale d'une irradiation aigüe, soit chez ceux qui ont été exposés à des doses plus faibles.

I - EFFET DES FORTES DOSES

Recherches dosimétriques liées aux effets à court terme

Les études hématologiques ainsi que l'étude des aberrations chromosomiques ont montré que pour les rayonnements gamma , l'étendue du dommage dépendait de la dose totale, du débit, de l'étalement dans le temps et du fractionnement.

Pour les neutrons, par contre, les effets observés dépendent beaucoup moins de la façon dont la dose est absorbée.

II - EFFETS SOMATIQUES A LONG TERME

1. Etude des cancers radio-induits

1.1. Observation des effets directs

En ce qui concerne les actinides, les travaux ont porté sur diverses formes de plutonium. Les aérosols ultra-fins se comportent comme des sels de plutonium de solubilité élevée.

L'étude des effets de l'Am 241 a montré que les premiers cancers osseux ont été observés chez des rats n'ayant fixé dans leur squelette que 100 picocuries par gramme d'os. Cette valeur ne représente que 2,5 fois la charge maximale osseuse chez l'homme.

Menées en collaboration avec le Laboratoire de Pathologie Pulmonaire de la COGEMA à Razès, les recherches sur les effets à long terme du radon ont montré qu'il était possible d'observer sur 500 animaux un excès de cancers du poumon avec une dose de 65 W.L.M.

Les études sur les irradiations globales neutroniques se sont poursuivies. Elles ont montré d'une part que le rôle du débit de dose était limité et, d'autre part, que chez le rat, l'efficacité

des neutrons était considérable.

Des lots d'animaux ont été irradiés avec des doses fractionnées de γ et avec une dose de rayons X délivrée en un temps très court (30 ns).

Toutes ces études visent à obtenir le maximum d'informations sur les cancers radio-induits du rat Sprague-Dawley. Les conditions d'irradiation sont variées, allant des irradiations globales neutroniques et photoniques, aux inhalations de radioéléments émetteurs α ou β en passant par les administrations locales d'actinides ou de produits de fission.

1.2. Etude des effets combinés de l'irradiation et de molécules chimiques

Plusieurs modalités d'irradiation ont été utilisées : irradiations neutroniques globales et inhalations de radioéléments émetteurs α (Radon 222, Plutonium 239). Plusieurs cancérigènes chimiques ont été associés (Benzopyrène, Méthylcholanthrène, Jaune de Beurre, Nitrosamines, etc..). On a administré aussi aux animaux, des molécules non cancérigènes (alcool, tabac, divers médicaments et la 5,6 Benzoflavone inducteur du Cytochrome P 450 pulmonaire). Enfin, des injections intra-pleurales de fibres minérales ont été pratiquées.

Une action synergique a souvent été observée. Elle porte sur le cancer spécifique dû à la molécule chimique et se traduit par un raccourcissement du temps de latence et une accélération de la vitesse du développement tumoral. En règle générale, l'action synergique ne se manifeste que si l'irradiation précède l'administration de la molécule chimique.

1.3. Etudes des mécanismes de contrôle de l'induction des cancers radio-induits

a/ Dans deux séries d'expériences portant sur 200 rats environ, nous avons pu mettre en évidence un effet contrôle à distance de la réaction inflammatoire aigüe sur la croissance et la dissémination des tumeurs radio-induites. Des études préliminaires nous ont montré que cet effet n'était pas dû à la surveillance immune.

b/L'un des mécanismes mis en jeu est l'activation des macrophages et des cellules tueuses,qui peut être envisagée comme facteur spécifique de la sensibilité individuelle pour la survenue des tumeurs radio-induites (recherche en cours). Un autre paramètre individuel semble être l'aptitude à la réaction inflammatoire.

c/ Pendant la phase de latence des tumeurs pulmonaires radio-induites, on n'observe pas de diminution significative de la réponse inflammatoire à distance du poumon. Cette diminution est par contre très caractéristique pendant la phase de latence des tumeurs induites par le benzo-a-pyrène.

d/ Les expériences antérieures ont mis en évidence l'étroite corrélation survenue entre des tumeurs radio-induites et le vieillissement des animaux. Les études entreprises ont visé à caractériser par un paramètre biologique le vieillissement cellulaire. Il a été observé que l'aptitude à former des colonies par les fibroblastes sous-cutanées était un bon index du vieillissement. Cet effet est uniquement observé lors des expériences de clonage. Il ne peut être mis en évidence dans les cultures en masse. Ce dernier résultat suggère fortement qu'un des facteurs

impliqués est membranaire et non génétique. Une irradiation générale induit un vieillissement accéléré dose-dépendant pour ce caractère.

e/ Enfin, il a été démontré en I979 que les organes irradiés secrétaient une ou plusieurs molécules augmentant in vivo les échanges de chromatides-soeurs dans les cellules de la moelle osseuse du rat. Ces études ont permis d'aboutir à des relations dose-effets cohérentes.

2. Irradiations des cellules germinales du rat

Les irradiations ont été pratiquées en utilisant soit des neutrons, soit des photons gamma . Elles montrent la très grande sensibilité du tissu germinal du rat aux irradiations chroniques et fractionnées ainsi que l'absence presque totale des phénomènes de restauration au niveau de ces cellules. Si en ce qui concerne les gammas, on observe un effet net du débit de dose, il n'en est pas de même pour les neutrons. Le débit de dose d'exposition le plus faible a été de 100 mR/h pour les gammas.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : OIO-N
Université de Leiden

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EFFETS GENETIQUES DES RADIATIONS IONISANTES SUR LES EUKARYOTES PLURICELLULAIRES
PROBLEMES, RESULTATS, PERSPECTIVES ET APPROCHES DE L'EVALUATION DU RISQUE GENETIQUE DE L'EXPOSITION AUX RADIATIONS IONISANTES

Cette monographie se propose d'analyser l'état des connaissances sur les effets génétiques des radiations ionisantes chez les eukaryotes pluricellulaires et de discuter l'évaluation du risque chez l'homme à la lumière des acquisitions récentes. Dans cet esprit, l'auteur procède à une synthèse des données de la littérature et cherche à dégager les implications pratiques des recherches actuelles pour enfin tenter de définir les champs d'investigation qui mériteront une attention particulière dans les années à venir.

La première partie du rapport est consacrée à l'étude du détriment génétique chez les eukaryotes pluricellulaires. La revue analytique s'intéresse successivement aux mutations génétiques chromosomiques (distinguant les modifications intragéniques et intergéniques), aux mutations géniques moléculaires (avec discussion des données expérimentales obtenues chez les Neurospora,la Drosophile, la souris, les mammifères dont l'homme), aux cellules en culture et aux plantes. Par ailleurs, les aberrations chromosomiques de structure et de nombre font l'objet d'une analyse complémentaire. La relation dose-effet est discutée de façon détaillée en étudiant notamment le rôle de divers paramètres physiques et biologiques susceptibles d'affecter le taux de mutation.

Dans une seconde partie, une étude des méthodes d'évaluation du risque lié à l'exposition aux radiations ionisantes est menée à partir d'une synthèse des connaissances relatives aux anomalies héréditaires spontanées, à l'incidence des anomalies chromosomiques ainsi qu'à toute donnée expérimentale. Les estimations des risques font l'objet d'une revue détaillée.

Dans une dernière partie, diverses orientations de recherche sont envisagées en vue d'améliorer l'évaluation des risques. Les diverses options suivantes sont proposées :

. Développement de méthodes expérimentales et mise au point d'indicateurs permettant de détecter les altérations induites par l'irradiation (mutations ponctuelles et aberrations chromosomiques).

. Explication des mécanismes de non-disjonction chromosomique et d'autres aberrations avec un effort particulier pour étudier la relation entre la structure chromosomique et le comportement.

. Etude des relations entre la radiosensibilité, la réparation génétique et les anomalies du processus de ségrégation.

. Enquêtes épidémiologiques portant sur la relation entre la dose reçue la fréquence des aberrations lymphocytaires en vue de faciliter l'interprétation des doses dans le cas des expositions non homogènes.

. Etudes portant sur des mammifères (dont les primates si possible) pour l'extrapolation quantitative des données à l'homme.

. Etudes visant à estimer les méthodes et les hypothèses qui entrent, dans l'évaluation des risques, dans l'extrapolation des cellules somatiques aux cellules germinales et des animaux d'expérience à l'homme.

. Etudes sur l'induction des mutations dans les cellules germinales et somatiques pour des doses et des débits de doses faibles, et sur la méthodologie de ces recherches.

. Identification et caractérisation (au niveau génétique et biochimique) des souches cellulaires de mammifères, qui présentent des sensibilités variables ou qui présentent des déficiences des mécanismes de réparation du DNA.

. Investigation détaillée de l'enzymologie des mécanismes de réparation du DNA dans des systèmes cellulaires de mammifères ainsi que de la relation entre les lésions radioinduites, les mutations et les aberrations chromosomiques.

. Etudes de la mutagénèse et du rôle des mécanismes structurels et induits de réparation dans les systèmes cellulaires de mammifères et dans d'autres systèmes qui se prêtent à ces études.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : OI6-I
Institut Mario Negri de Milan

Responsable scientifique : A. TOGNONI

ETUDE BIBLIOGRAPHIQUE ET ANALYSE CRITIQUE DES DONNEES RELATIVES AUX EFFETS CANCEROGENES DES RADIATIONS IONISANTES CHEZ L'HOMME

Cette étude consiste en une revue analytique des données de la littérature récente afin d'actualiser les connaissances relatives à l'évaluation des effets des radiations ionisantes sur l'homme et de faire le point des nouvelles acquisitions publiées depuis le dernier rapport de l'U.N.S.C.E.A.R.

Les cinq sujets suivants ont été abordés :

1. Etude des effets sanitaires chez les travailleurs de Hanford

Une discussion de résultats de cette étude est menée à la lumière des nombreux commentaires qu'elle a suscités. L'analyse se propose d'examiner la place qui doit être reservée aux conclusions de l'étude en fonction des limites inhérentes à la méthode utilisée.

2. Tumeurs radio-induites chez les enfants

L'étude d'Oxford représente une somme importante de renseignements dont les implications pour la radioprotection doivent être discutées en fonction de la méthode analytique.

3. Tumeurs induites par la radiothérapie

De nombreuses informations sont disponibles dans ce domaine : études réalisées chez les patients atteints de spondylarthrite ankilosante, étude des tumeurs du sein après radiothérapie pour affection mammaire bénigne, et étude de l'incidence des tumeurs chez les sujets irradiés pour teigne du cuir chevelu ...

4. Effets des explosions nucléaires

Ce volet de la revue s'intéresse aux dernières données publiées sur la population exposée à Hiroshima et Nagasaki ainsi qu'aux populations américaines soumises aux retombées radioactives.

5. Tumeurs osseuses après radiodiagnostic

La discussion porte essentiellement sur les arguments

utilisés par les différentes parties en controverse sur le sujet.

Enfin, la discussion est axée sur les problèmes méthodologiques posés par ce type d'étude en insistant notamment sur la nécessité de trouver des échantillons de taille suffisante pour que l'analyse soit effectuée dans les meilleures conditions de puissance statistique. La signification des publications récentes est critiquée en fonction de considérations purement épidémiologiques telles que la possibilité de rejeter l'hypothèse nulle quand elle est vraie ou encore des difficultés de l'interprétation statistique tenant à la grande variabilité des intervalles de confiance selon la puissance statistique des estimations Une discussion est consacrée aux deux modèles de cancérogénèse actuellement développés (modèle linéaire et modèle quadratique) qui, probablement, ne sont pas incompatibles mais peut-être applicables à des formes de cancer différentes.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : OI7-EIR
The Medical Social Research Board de Dublin

Responsable scientifique : G. DEAN.

ETUDE DES EFFETS A LONG TERME DE L'IRRADIATION THERAPEUTIQUE DE LA TETE ET DU COU POUR ACNE

Ce programme consiste en une étude de prévalence de certains cancers qui porte sur une cohorte de IO 000 sujets irradiés pour acné de la tête et du cou.

En effet, MODAN et coll. ont montré que, chez IO 000 enfants traités pour teigne entre I949 et I960, le groupe irradié présente un risque significativement plus élevé d'affections malignes et bénignes de la tête et du cou, notamment du cerveau, de la parotide et de la thyroïde. Cette observation suggère une relation dose-effet pour des niveaux de dose relativement faibles et demande donc à être confirmée ou infirmée par de nouvelles enquêtes épidémiologiques. Or, certaines affections de la face ont été traitées pendant de nombreuses années par l'administration de rayons X mous. Les dossiers d'irradiation et l'étude du suivi des patients fournissent les deux catégories de données entre lesquelles une relation est recherchée.

Le programme comprend trois parties :

I - COLLECTE DES DONNEES

Les dossiers des sujets ont été obtenus dans sept hôpitaux au Royaume-Uni :

. Liverpool : 4 253 patients traités depuis 1923 au Liverpool Royal Infirmary et au Liverpool Radium Institute ont été identifiés.

. New-castle : 1 116 dossiers ont été obtenus au Royal Victoria Infirmary et au New-castle General Hospital.

. Manchester :1 687 dossiers proviennent du Skin Hospital.

. Birmingham :Plus de 1 200 dossiers proviennent du General Hospital.

. Glasgow : Près de 3 000 dossiers ont été obtenus au Western Infirmary.

. Leeds : Environ 3 000 dossiers proviennent du General Infirmary.

. London :300 dossiers ont été obtenus au St John's Hospital.

Les informations portées sur les fiches ou les dossiers sont très variables.

L'ensemble des dossiers atteint le nombre de 14 531 patients. Ce chiffre comprend environ 12 000 acnés vrais.

II - SUIVI DES PATIENTS

A l'aide du National Health Service Register de Southport, il est possible de retracer et d'analyser le devenir de IO 000 sujets environ. La cause des décès peut être obtenue par le certificat de décès.

Par ailleurs, les registres régionaux et le Registre National du cancer, lequel existe depuis I971, sont consultés. Les informations portant sur la mortalité et la morbidité seront analysées au Department of Health and Social Security, à l'intérieur d'un programme qui permet de les traduire dans les termes de l'ICD (International Classification of Deceases, Traumatisms and Causes of Death-(OMS)). Le but de cette opération est bien entendu de pouvoir, en dernière analyse, comparer les taux de cancers obtenus avec ceux de la population générale.

III - ETUDE DOSIMETRIQUE ET ANALYSE DES RESULTATS

Les dossiers d'irradiation des patients comportent toutes les informations utiles pour calculer de façon précise la dose reçue par les différents tissus et organes : dates de radiothérapie, voltage, filtre, surface du champ, temps d'exposition, ..

Leur analyse montre que les techniques d'irradiation varient beaucoup d'un hôpital à l'autre. Par exemple, à Leeds, on appliquait en général 3 champs de 450 R chacun sous 65 KV. A Birmingham, la dose était de 600 R (300 à 1200 R) par champ sous 65 KV. A Glasgow, la dose était en moyenne de 1500 R (900-1900 R) sous 140 KV (avec filtres aluminium et cuivre). Il y a donc un facteur de 3 entre la dose d'exposition la plus faible et la plus forte et ce facteur dépasse 3 en termes de dose tissulaire absorbée.

Le Dr Ellis (Leeds) procède, selon la méthode utilisée par lui-même pour l'enquête de Doll sur les spondylites rhizoméliques; une estimation des moyennes de dose d'exposition est effectuée sur des sous-échantillons. On calcule ensuite la relation dose-effet entre la moyenne de la dose appliquée et l'incidence des cancers du cerveau, des glandes salivaires, de la thyroïde et des leucémies.

Les résultats, la discussion et les conclusions seront présentés dans le rapport final.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 018-099-79-10-PSAF
Centre d'Evaluation de la Protection dans le domaine Nucléaire (CEPN) Fontenay-aux-Roses

Responsable scientifique : F. FAGNANI

EVALUATION DES REJETS ET DES COUTS ASSOCIES AUX DIFFERENTS SYSTEMES DE TRAITEMENT DES OXYDES DE SOUFRE DES CENTRALES THERMIQUES CLASSIQUES

La recherche de critères d'acceptabilité en vue d'une gestion cohérente des risques industriels d'origines différentes requiert la connaissance des niveaux de risques résiduels associés aux différentes politiques de protection envisageables ainsi que des coûts de protection correspondants.

L'étude a déterminé pour les centrales thermiques au fuel et au charbon le coût et l'efficacité de divers systèmes de traitement utilisés, envisagés ou envisageables réduisant les rejets de poussière et/ou d'oxydes de soufre. L'efficacité est exprimée en pourcentage d'émission.

Dans un premier temps, nous avons élaboré un modèle simplifié de rejet pour une centrale au fuel ou au charbon de 1 GWe. Ce modèle permet de déterminer en fonction des caractéristiques du combustible utilisé et du taux de polluant rejeté la quantité annuelle de cendres et de dioxyde de soufre émise par la centrale et ce, pour chaque système de traitement.

Parmi les nombreux systèmes cités dans la littérature, nous n'avons retenu que ceux qui paraissaient les plus réalistes et dont nous pouvions connaître l'efficacité en termes de réduction de rejet et de coût . Ainsi, avons nous envisagé pour la centrale au charbon, cinq systemes de réduction des poussières, et quatre pour les oxydes de soufre. Pour la centrale au fuel,on dispose de quatre systèmes pour les poussières et six pour les oxydes de soufre.

Ceci a permis de déterminer pour chaque système de protection un ratio coût-efficacité traduisant le coût associé à la réduction d'une tonne de poussières et/ou de dioxyde de soufre.

Ceci nous a permis de hiérarchiser les différents systèmes envisagés. Nous avons obtenu les résultats partiels suivants :

Centrale au charbon :

Poussières :

1. Utilisation d'additifs	4,7 F	par tonne de poussière retenue
2. Dépoussiéreur mécanique	4,9 F	" "
3. Dépoussiéreur électrostatique	9 F	" "

Dioxyde de soufre :

1. Lavage du charbon	1300F	par tonne de SO_2 piégée
2. Epuration à la chaux	5400F	"
3. Procédé EDF-IFP	7200F	"

Centrale au fuel :

Poussières :

1. Dépoussiéreur mécanique	500 F.	par tonne de poussière retenue
2. Dépoussiéreur électrostatique	900 F	" "
3. Utilisation d'additifs	3500 F	" "

Dioxyde de soufre :

1. Valorisation des fuels lourds	1000 F	par tonne de SO_2 piégée
2. Utilisation de TBTS (10%)	1300 F	"
3. Injection de chaux	2300 F	"

La base de résultats ainsi obtenus permettrait de calculer, dans une étude ultérieure, les rapports coût-efficacité des systèmes avec un indicateur d'efficacité exprimé en termes d'effets sanitaires, mieux adapté à des comparaisons avec d'autres secteurs industriels.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 019 F
Ecole Nationale de Santé Publique de Rennes
Association Villermé

Responsable scientifique : L. MASSET

ETUDE DE L'IRRADIATION NATURELLE EN BRETAGNE

Cette étude s'inscrit dans le cadre des recherches méthodologiques d'évaluation de l'exposition de la population européenne aux radiations ionisantes et répond, pour ce qui la concerne, à une tentative d'élaboration d'un bilan d'exposition à l'irradiation d'origine naturelle. L'objectif principal est de chercher à définir des protocoles de mesure adéquats pour évaluer l'irradiation naturelle à l'échelle de la région et à établir un indice représentatif de la dose population correspondante. La Bretagne constitue une zone pilote intéressante par la diversité géologique des sols et par la prépondérance des terrains granitiques.

I - EVALUATION DE L'EXPOSITION EXTERNE

1.1. Mise au point d'une méthode de mesure

Divers procédés de mesure sont testés en vue d'obtenir une évaluation systématique et rapide de l'activité naturelle sur un territoire étendu.

. L'enregistrement manuel de l'activité tellurique en milieu naturel, défini par l'absence de toute perturbation artificielle du terrain, est insuffisant à plusieurs titres (faible densité de mesure du fait d'une mise en oeuvre lourde, absence de prise en compte de tous les paramètres d'environnement ..) ; il reste cependant indispensable dès lors qu'il s'agit de faire intervenir des critères géologiques dans l'analyse et qu'il s'agit de disposer d'une base de référence pour juger de protocoles plus élaborés.

. L'enregistrement automatique en continu sur route permet d'augmenter de façon significative la densité des relevés et d'uniformiser les conditions de la mesure (MAB 601 couplé à un enregistreur - standardisation des données par rapport à un RSS 111).

1.2. Résultats généraux

. L'analyse d'environ 6000 mesures réparties dans 230 communes (soit approximativement 1/5 de la Bretagne) met en évidence les principaux résultats suivants :

. La valeur moyenne du rayonnement cosmique, mesuré en mer, est de 4,1 ± 0,7 µrad/h.

. Les niveaux d'activité les plus faibles (4-6 µrad/h) correspondent aux zones inhabitées et les valeurs plus élevées (supérieures à 8,5 µrad/h) correspondent aux zones habitées et aux affleurements granitiques. Certaines variables de l'environnement, tels que les lieux de passage ou les points d'eau, semblent au contraire faiblement corrélées avec la radioactivité.

. Le niveau moyen d'activité du milieu naturel (moyenne = 9 ± 2,7 µrad/h) est inférieur à celui des routes (moyenne = 11 ± 3,5 µrad/h), lui-même inférieur à celui du milieu urbain (moyenne =12,1 ± 3,9 µrad/h) où la distribution des valeurs est plus étalée.

. Fait notable, l'étude de la distribution des valeurs dans les communes suggère que la moyenne des relevés enregistrés dans chaque environnement de référence (naturel, routier, urbain) est statistiquement représentative. Il semble donc possible de caractériser la commune par 3 valeurs au moins.

. La prise en compte des paramètres géologiques souligne la nécessité d'approfondir l'étude pour confirmer ou infirmer la validité de la mesure sur route et pour chercher à établir des protocoles d'évaluation simplifiés.

II - EVALUATION DE L'IRRADIATION DOMESTIQUE

L'étude d'un lot restreint d'habitations dans des conditions standardisées (mesures ponctuelles répétées à un mois d'intervalle dans des pièces closes depuis 12 heures au moins) met en évidence des concentrations de radon très variables selon les constructions, et parfois élevées (jusqu'à 39 pCi/l d'air). En l'état actuel des travaux, l'importante fluctuation temporo-spatiale des valeurs mesurées n'a pas pu être mise en relation avec les matériaux de construction ou les conditions climatiques.

CONCLUSION

Cette étude décrit de façon relativement approfondie l'exposition externe en Bretagne. Elle souligne la nécessité de poursuivre les investigations pour évaluer l'irradiation d'origine domestique. Diverses orientations sont envisagées à cet égard (mesure de l'exposition externe dans les constructions, intercomparaison de l'enregistrement scintillométrique avec celui du dosimètre , poursuite des mesures de radon en utilisant notamment un prélèvement continu).

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 020-UK
St George's Hospital Medical School de Londres

Responsable scientifique : T.E. BENNETT

ETUDE DE MORTALITE EN RELATION AVEC LES IRRADIATIONS DIAGNOSTIQUES ET LE TABAC

Le problème étudié est celui de l'existence d'une synergie entre les relations ionisantes et le tabac dans l'induction des affections respiratoires chez l'homme.

Cette étude porte sur une cohorte rétrospective de sujets mâles, employés d'usines de la région londonienne, qui avaient fait, en 1960, l'objet d'une expérimentation visant à déterminer l'efficacité du dépistage radiologique précoce du cancer du poumon. Les informations les concernant, nom, adresse, âge, employeur, lieu de l'emploi, médecin traitant, radiographies précédentes, affections respiratoires et consommation de tabac avaient été notées dans le dossier initial. Trois cohortes, chacune de 30 000 sujets, avaient été étudiées. Deux cohortes avaient été l'objet de radiographies du thorax tous les 6 mois pendant 3 ans, alors que la troisième cohorte n'en avait subi que deux, une à l'entrée et une 3 ans après. La distribution des âges au départ de l'étude en 1960 était uniforme de 45 à 64 ans.

Tous les dossiers sont disponibles ainsi que, pour l'une des cohortes, les films de 1963 jusqu'aujourd'hui.

Dans un premier temps, il a été nécessaire de procéder à une informatisation des données de base et à une étude de la distribution des activités professionnelles. Les 27 000 sujets actuellement répertoriés représentent 6000 professions différentes, regroupées en 200 catégories (correspondant à celles de l'Office of Population and Census Survey). Il est ainsi possible de classer la population par niveaux socio-économiques.

Tous les sujets sont des employés d'usines de la région londonienne et n'ont été exposés à aucun risque professionnel connu (chimique, respiratoire, ..). Il s'agit de travailleurs de l'industrie aéronautique et électronique.

L'étape suivante consistant à rassembler les données supplémentaires, relatives à l'exposition aux irradiations diagnostiques, à l'identification des personnes et dans certains cas, au suivi de leur passé au cours des vingt années écoulées, a été mise en place.

Le suivi sera fait selon la séquence : employeur, médecin traitant, Comité du General Practitionners, dernière adresse connue et Registre Central du National Health Service à Southport permettant notamment de connaître les causes de décès.

D'autres sources d'information seront éventuellement utilisées, comme les listes électorales, registres hospitaliers, registres du cancer, dossiers de retraites ... Des listings rassembleront toutes les

informations dont l'analyse a pour but de déterminer les risques de mortalité pour des affections spécifiques liées au tabac ou à d'autres facteurs isolés ou combinés.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 021-099-79-IO-PSAF
Centre d'Etudes sur l'Evaluation de la Protection dans Domaine Nucléaire (CEPN),Fontenay-aux-Roses

Responsable scientifique : F. FAGNANI

RECHERCHE DES VALEURS IMPLICITES DU DETRIMENT SANITAIRE

Cette étude s'inscrit dans un projet général de comparaison des efforts de protection dans l'industrie électronucléaire et divers autres secteurs d'activité.

Le secteur étudié est celui de l'industrie de l'amiante dans la mesure où ce produit conduit à un des risques professionnels les plus importants.

L'amiante est, d'autre part, une des rares substances nocives pour lesquelles on dispose de données relatives aux relations " dose-risque " ; celles-ci ont été obtenues essentiellement à travers des enquêtes épidémiologiques menées auprès de travailleurs.

L'étude comporte deux volets principaux. Le premier concerne l'évaluation des risques asbestosiques.

On y rappelle les diverses sources et conditions d'exposition au risque.

On évoque ensuite la complexité de la pathologie de l'amiante et l'hétérogénéité des effets (fibrosants et cancérogènes : asbestose et cancers broncho-pulmonaires, mésotheliomes, etc...) ; les caractéristiques spécifiques des affections dues à l'amiante telles que : effets différés à long terme, maladies évolutives, non spécificité de certains cancers, difficultés de diagnostic, etc... sont passées en revue afin de souligner le caractère incertain de la mesure du risque.

On aborde ensuite la question des relations exposition-risque. On ne considèrera que les risques léthaux. Pour le cancer broncho-pulmonaire, les travaux publiés font état de diverses relations exposition-risque linéaires sans seuil. On en a retenu plusieurs afin de disposer d'une fourchette reflétant les incertitudes de la quantification. Pour les mésothéliomes, on a retenu un modèle suggéré par Julian Peto et al. lequel dépend du temps écoulé depuis la première exposition.

Pour la mortalité associée à l'asbestose, il existe plusieurs modèles dépendant du temps de séjour des fibres dans les alvéoles pulmonaires. Nous avons retenu ici encore les hypothèses extrêmes afin de fournir des fourchettes de résultats.

Le second volet de l'étude concerne l'évaluation des valeurs implicites de l'effet évité.

On y présente un bref aperçu de la règlementation actuelle, laquelle a entraîné une réduction des empoussièrements dans les usines utilisant l'amiante.

Les données concernant l'efficacité des mesures de réduction de l'empoussièrement et le coût de celles-ci sont très lacunaires. Il a été possible cependant de procéder, dans le cas français, à une évaluation grossière de la réduction des niveaux d'exposition, ainsi que des coûts associés pour des usines d'amiante manufacturé (textiles, garnitures de frein). Pour un effectif global de 2 à 3000 employés, la réduction des empoussièrements est en moyenne de 1,5 à 2,5 fibres/cm3 et les coûts s'élèvent à plusieurs dizaines de millions de francs. A partir des données relatives à la réduction des niveaux d'empoussièrement, on a calculé,compte tenu des effectifs, un ordre de grandeur du risque sanitaire évité, ce qui nous a permis de tirer des fourchettes de valeurs implicites de l'effet évité. On a mis en perspective ces valeurs avec les données correspondantes calculées sur les informations provenant de la littérature internationale.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 023-099-79-IO-PSAF
Centre d'Evaluation de la Protection dans le domaine Nucléaire (CEPN) Fontenay-aux-Roses

Responsable scientifique : F. FAGNANI

OPTIMISATION DE LA PROTECTION DANS LE CAS DES TRAVAILLEURS - ETUDE EXPLORATOIRE GENERALE ET APPLICATION A L'EXTRACTION DU MINERAI D'URANIUM

Deux rapports distincts ont été rédigés. Le premier concerne une étude exploratoire de faisabilité relative à l'optimisation des expositions professionnelles dans le cadre du fonctionnement normal du cycle du combustible PWR. Le second rapport a trait à l'optimisation des expositions professionnelles dans une mine d'uranium.

L'étude de faisabilité détermine tout d'abord les niveaux de risque individuel et collectif pour chacune des installations du cycle du combustible. Ce travail a été effectué à partir d'une synthèse des données françaises et internationales.

La comparaison est menée pour un cycle à l'équilibre normalisé à production annuelle d'énergie nette de 5,6 TWh (énergie liée au fonctionnement d'un réacteur de 1 GWe durant 5600 h).

Il en ressort que les maillons du cycle présentant les risques les plus élevés (individuel et/ou collectif) sont l'extraction du minerai, le réacteur et le retraitement. Les déterminants de ces niveaux de risque ont été explicités (radon pour les mines, vieillissement des structures des réacteurs, problèmes de conception entraînant des difficultés lors de l'exploitation et de la maintenance des équipements).

La deuxième partie du rapport concerne les perspectives de réalisation d'études d'optimisation.

En ce qui concerne le retraitement, l'absence de données ne permet pas d'envisager pour le moment des études concrètes. Pour les mines, la distinction est faite entre l'extraction filonienne et les gisements sédimentaires. Dans les deux cas, il convient d'approfondir les connaissances relatives à l'évaluation de l'exposition (Problème de la conversion WL/Sv) et mettre au point des modèles de simulation de la ventilation. Enfin, pour les réacteurs, deux axes de recherche sont proposés, d'une part, l'explicitation des interactions entre protection et exploitation, d'autre part, la mise au point d'une base de données

dosimétriques associant aux débits de dose le temps d'intervention par type d'opération.

Le second rapport présente un modèle théorique général de l'exposition externe et de la contamination α dans une mine filonienne. Ce modèle tend à expliciter les paramètres principaux intervenant dans le calcul de ces deux modalités d'exposition (teneur en uranium dans les chantiers, dans les zones hors chantier, caractéristiques liées à la géométrie des zones, temps de séjour dans les diverses zones, débits d'aérage primaire et secondaire. Aussi bien pour l'exposition externe que pour la contamination α , le modèle théorique détermine l'engagement d'équivalent de dose collective effective. Il permet de plus d'analyser les paramètres fondamentaux du risque.

On a procédé ensuite à une discussion des principales actions envisageables pour réduire l'une ou l'autre des modalités d'exposition. La connaissance des coûts associés à ces actions permettrait d'effectuer une analyse coût-efficacité mettant en balance notamment les coûts de protection envisageables et la réduction du risque travailleurs qui en résulte. En fonction de la valeur de l'homme-Sievert travailleur, il serait alors possible de procéder à une étude d'optimisation du risque professionnel conformément aux recommandations de la CIPR.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 024 F
Centre d'Evaluation de la Protection dans le domaine Nucléaire (CEPN) Fontenay-aux-Roses

Responsable scientifique : F. FAGNANI

ETUDE DES TRANSFERTS DE RADIONUCLEIDES A TRAVERS LES CIRCUITS DE DISTRIBUTION (CAS DES VIANDES NON BOVINES, DE L'ALIMENTATION ANIMALE, DES PRODUITS IRRIGUES)

Ces études ont pour but de déterminer les contaminations spécifiques et globales des produits livrés à la consommation, connaissant les niveaux de contamination au stade de la production.

On modélise le transfert d'un radionucléide donné à travers les circuits de distribution des produits en fonction des échanges et des transformations subis. A partir des données statistiques disponibles, on estime sous certaines hypothèses économiques les flux d'échanges inter-régionaux. La connaissance des tableaux d'échanges permet alors de calculer les transferts de contamination.

Au cours des années précédentes, on a successivement abordé les problèmes des céréales, des produits laitiers, des produits de la mer, des viandes bovines.

- Viandes non bovines :

Les statistiques recueillies au cours de cette dernière étude ont montré que la part prise par chacun des pays de la Communauté dans la production et les échanges varie beaucoup selon les viandes considérées, de même que les consommations moyennes. En raison de la part importante prise par la viande porcine en R.F.A. et en France, on a décidé d'étudier les échanges au niveau régional pour ces deux pays. La viande de volaille mérite aussi une analyse détaillée, dans le cas de l'Italie et de la France.

Les données statistiques exploitées concernent l'année 1977. Les hypothèses de contamination portent sur le Cs 137. Les activités ingérées annuellement sont extrêmement faibles et sont plus élevées à partir de la consommation de viande de porc qui est la viande la plus consommée en Europe. Les échanges de produits ont pour effet de transmettre, en partie, la contamination de la viande de porc du Danemark vers le Royaume-Uni, ainsi que des Pays-Bas à l'Italie. Au niveau régional, on note en Allemagne un transfert sensible du Niedersachsen vers plusieurs régions.

Pour la volaille, le transfert le plus notable entre pays se fait des Pays-Bas vers la R.F.A.

- Alimentation animale :

L'étude de l'alimentation animale est le complément logique des précédentes. Elle met à profit les données déjà recueillies à l'occasion des recherches sur les échanges de produits laitiers, céréaliers et marins.

Le premier volet de l'étude développe plus particulièrement les aspects techniques de l'alimentation animale :

besoins des différentes espèces animales
apports disponibles
rations alimentaires ...

Les rations types retenues permettent d'évaluer les activités ingérées par les différentes espèces animales : volaille, porçins, bovins, ovins ..., à partir des concentrations de radioéléments dans les matières premières utilisées, et par conséquent, la part de contamination transférée à l'homme par suite de la consommation de produits animaux.

- Légumes irrigués :

Le but de la recherche est de déterminer l'influence des échanges et l'importance de l'irrigation sur la contamination moyenne des individus de la C.E.E. divisée en 25 zones géographiques.

Après une analyse économique des circuits et des flux, le recueil des données sur les productions, les consommations et les échanges d'une part, et l'estimation des statistiques inconnues d'autre part, ont permis de construire un modèle de transfert des contaminations qui prend en compte les taux d'irrigation régionaux, les différentes voies de transfert à la plante et à l'homme.

Il ressort de cette étude que l'Italie centrale et méridionale sont les seules régions qui, par l'importance des exportations et de l'irrigation effectuée, étendent le risque de contamination aux principaux pays importateurs de la C.E.E. notamment à la RFA et dans une moindre mesure à la France, en cas d'une présence éventuelle de radioéléments en quantité importante dans les eaux d'irrigation.

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 025 F
Hôpital Necker de Paris

Responsable scientifique : J.L. FUNCK-BRENTANO

COMPARAISON DES LESIONS INDUITES AU NIVEAU DU REIN CHEZ LE RAT ET CHEZ L'HOMME PAR LES RADIATIONS IONISANTES

Cette étude entre dans le cadre d'une tentative d'explication de la pathologie humaine par un modèle animal :

I - ETUDE DE L'ANIMAL

1.1. Lésions non tumorales

Dans un travail préliminaire, il est apparu que la fréquence des néphrites spontanées variait considérablement en fonction de la souche de rats étudiée. Après 2 ans d'élevage, tous les animaux Sprague Dawley étaient macroscopiquement porteurs de lésions rénales à type de néphrite associant des dilatations kystiques des segments distaux à une glomérulonéphrite. Chez le rat Wag, la fréquence des anomalies macroscopiques est considérablement plus faible et le tableau lésionnel est incomplet. Il semble donc qu'une prédisposition génétique du tissu rénal soit essentiellement à l'origine des différences observées dans les deux lignées.

Dans la lignée sensible, les lésions rénales sont détectées plus précocement lorsque les animaux sont soumis à une irradiation interne chronique consécutive à l'élimination urinaire et à l'inhalation de radionucléides émetteurs α (plutonium, américium, curium). Dans la lignée non sensible, la cause de la mort des animaux semble le plus souvent liée à des pathologies entrant dans le cadre nosologique des maladies autoimmunes.

1.2. Lésions tumorales

Dans la lignée sensible, des tumeurs rénales bénignes et malignes de nature histologique diverse, sont observées avec une fréquence de 0,5 pour mille en cas d'irradiation et qui atteint 40% dans le cas d'irradiation combinée avec le tabac.

Dans la lignée non sensible, un seul cas de tumeur a été observé mais les doses délivrées au rein ont été beaucoup plus faibles.

Au total, il convient de souligner la variabilité des types tumoraux ainsi que la fréquence relativement élevée des néphroblastomes chez le vieux rat indicatrice de lésions dysgénésiques de l'organe.

II - ETUDE DE L'HOMME

L'analyse se propose de vérifier si les lésions observées dans les maladies auto-immunes sont comparables à celles observées chez le rat et notamment à celles qui suggèrent une réaction auto-immune après contamination par les émetteurs α .

Certaines maladies systémiques avec atteinte rénale peuvent réaliser chez l'homme un aspect lésionnel comparable aux maladies auto-immunes. Dans le syndrome de Sjögren, l'image histologique est tout à fait comparable à celle que l'on a observée après irradiation chez le rat Sprague Dawley et à un degré moindre chez le rat Wag.

Considéré comme le résultat d'un désordre immunologique d'origine lymphocytaire, le syndrome de Sjögren est souvent traité par les corticoïdes ou les immuno-suppresseurs. Des rats ont donc été soumis à un traitement immuno-suppresseur par l'Azathioprine ou à une thymectomie néonatale ou encore à une immuno-stimulation par le B.C.G. pour vérifier l'action de ces traitements sur le développement des lésions rénales.

L'étude conclut que si l'irradiation entraîne des lésions rénales proches de celles observées au cours du syndrome de Sjögren, l'induction d'une immuno-dépression ou au contraire d'une immuno-stimulation n'entraîne pas de modification appréciable des lésions.

Association EURATOM-CEA 099-76-I-PSAF

SOUS-CONTRAT N° : 026 F
Département de Chimie Appliquée et d'Etudes Analytiques,
Service d'Etudes Analytiques, CEA, Fontenay-aux-Roses

Responsable scientifique : A. REGNAUD

ETUDE DES REACTIONS DE DIFFERENTES FORMES PHYSICO-CHIMIQUES DU PLUTONIUM EN PRESENCE D'EAU DE MER

L'étude a porté sur Pu(VI).

L'eau de mer nous est très vite apparue être un milieu trop complexe pour être utilisée immédiatement dans les expériences projetées.

Nous avons travaillé avec une eau de mer simplifiée (S1) contenant NaCl 0,546 M (31,91 gl^{-1}), $NaHCO_3$ 2,3 10^{-3} M (0,19 gl^{-1}), le pH mesuré est de 7,70. Les solutions sont faites à partir de l'eau permutée puis bidistillée du laboratoire.

Nous avons choisi d'introduire des micro-volumes d'une solution concentrée de plutonium de façon à modifier le moins possible le milieu.

Le plutonium est suivi par spectrophotométrie d'absorption dans le visible et le proche infra-rouge. Nous n'entrons pas dans la discussion des spectres obtenus : celle-ci devrait essentiellement faire l'objet d'un rapport séparé. Cependant, les renseignements qu'ils fournissent sont condensés avec d'autres éléments et utilisés dans le tableau ci-joint, résumé des essais effectués.

Nous avons été amenés à étudier séparément l'influence des constituants de S1), à savoir l'eau seule, les carbonates, les chlorures et à suivre en fonction du temps l'évolution de ces différentes solutions (voir tableau).

La conclusion principale à laquelle nous aboutissons est l'existence de Pu(VI) sous forme d'une espèce carbonatée dans (S1). Cependant, il semble que cette espèce disparaisse lentement* (à l'échelle du laboratoire) au profit d'un hydroxyde insoluble avec établissement d'un équilibre. Le facteur temps joue donc un rôle très important et il nous paraît primordial de suivre l'évolution de certaines solutions (pH, spectres d'absorption, apparition ou disparition de phases solides) sur des périodes de temps d'au moins six mois pour pouvoir décrire de façon plus sûre le comportement du plutonium.

* quelques mois.

MILIEU	OBSERVATIONS	REMARQUES
EM(S1) NaCl 0,546 M $HNaCO_3$ 2,3 IO^{-3} M	50 ml EM(S1) + 5 µl Pu(VI) (1)	
	Evidence Pu(VI) par apparition d'une déformation dans le spectre vers 850 nm	C_o (2) = 2,5 IO^{-5} M pH 7,45
	50 ml (S1) + 50 µl NaOH M + 50 µl Pu(VI)	
	pH 7,95 puis 7,70 (8j) et 7,50 (2 mois) Evidence Pu(VI) 850 nm Absence PuO_2^{2+} 832 nm	Co = 2,5 IO^{-4} M
	50 ml (S1) + 250 µl NAOH M + 250 µl Pu(VI)	
	pH 7,60 puis 7,50 (2 mois) Evidence Pu(VI) Un précipité apparaît au bout de 2 mois	Co = 1,25 IO^{-3} M Pu(VI) décroît en fonction du temps
$NaHCO_3$ 2,3 IO^{-3} M	50 ml $NaHCO_3$ 2,3 IO^{-3} M + 250 µl NaOH I M + 250 µl Pu(VI)	
	Evidence Pu(VI) complexé pH 7,9 puis 8,3 (2 1/2 mois) Apparition d'un précipité au bout d'1,5 mois) Disparition presque totale du pic d'absorption de Pu(VI), au bout de 2 1/2 mois	Complexe(s) → Pu(VI) Pu(VI)-carbonates hydroxidé Précipité vert de plus en plus abondant
H_2O dégazée	50 ml H_2O dégazée à l'argon + 40 µl Pu(VI)	
	PuO_2^{2+} (832 nm)	pH 3,40 apparemment PuO_2^{2+} est la seule espèce présente
	50 ml H_2O dégazée + 250 µl NAOH I M + 250 µl Pu(VI)	
	Précipité de plutonium qui se redissout partiellement en fonction du temps (équilibre ions ⇄ hydroxydes ⇄ pH pH (15j) 4,60 Evidence PuO_2^{2+} (832 nm)	Le pH diminue rapidement en raison de l'hydrolyse pas de complexes
NaCl 0,55 M eau dégazée	50 ml NaCl 0,55 M + 85 µl NaOH 1 M (exempt de carbonate) +100 µl Pu(VI)	
	Précipité vert abondant immédiat pH 5,6 (15j) - aucune indication donnée par les spectres d'absorption	pH instable (hydrolyse) absence complexes chlorure

(1) Solution de Pu(VI) : 0,25 M en $HClO_4$ ≃ 1 N
(2) Concentration initiale du plutonium dans la solution

Document 2

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N°: 027-F
Département de Protection, Service d'Etudes et Recherches sur l'Environnement, Laboratoire des Transferts Atmosphériques, CEA, Fontenay-aux-Roses

Responsable scientifique : C. CAPUT

EFFET DE L'HUMIDITE ATMOSPHERIQUE SUR LA CAPTATION DE L'IODE ELEMENTAIRE PAR LES VEGETAUX

Les travaux effectués jusqu'ici sur la captation de l'iode élémentaire par les végétaux concluaient généralement à un taux de sorption directement relié au taux de diffusion gazeuse à travers les stomates, l'humidité n'intervenant qu'au deuxième degré, en tant que paramètre agissant sur l'ouverture stomatique. Vingt expériences ont été réalisées dans une chambre d'exposition sur des plants de haricot en pots dans des conditions contrôlées d'humidité, de température , de vitesse de l'écoulement, d'éclairement et de résistance foliaire de transfert à la vapeur d'eau. Lors de chaque essai de 6 heures, 6 feuilles ont été prélevées à intervalles réguliers pour permettre d'accéder à la cinétique de captation, les concentrations dans l'air étant mesurées avec la même fréquence. La concentration en iode était inférieure à 0,1 $\mu g\ m^{-3}$ d'air, marqué à l'isotope 132. Dans tous les cas la cinétique de captation est représentée par une droite, ce qui implique une vitesse de dépôt constante, donc l'absence de saturation.

Dans le cas d'une humidité relative faible (30%), les valeurs des vitesses de dépôt obtenues ont été portées en fonction de l'inverse de la résistance stomatique sur le graphique de la figure 1. On observe que la droite obtenue est en bon accord avec la relation

$$V_d = \frac{0,32}{r_s} \qquad (1)$$

que l'on peut déduire en supposant que le transfert de l'iode se fait exclusivement à travers les orifices stomatiques. Le coefficient 0,32 qui apparaît dans cette relation est le rapport des coefficients de diffusion moléculaire de l'iode et de l'eau (0,08 $cm^2\ s^{-1}$ et 0,25 $cm^2 s^{-1}$ respectivement).

Lorsque l'humidité relative devient plus importante (voir fig. 2), la vitesse de dépôt obtenue est supérieure à celle que l'on pourrait déduire de la relation (1) ce qui prouve que l'iode n'est pas absorbé exclusivement par voie stomatique, mais qu'un mécanisme d'adsorption superficielle intervient. Ainsi par exemple dans le cas d'une humidité relative de 80% et d'une résistance stomatique pour la vapeur d'eau de 2,5 $s.cm^{-1}$, la vitesse de dépôt d'iode par voie stomatique calculée par la relation $V_d = 0,32/r$ ne représente que 10% environ de la vitesse de dépôt mesurée. Il apparaît donc clairement que l'humidité relative constitue le paramètre principal conditionnant la captation.

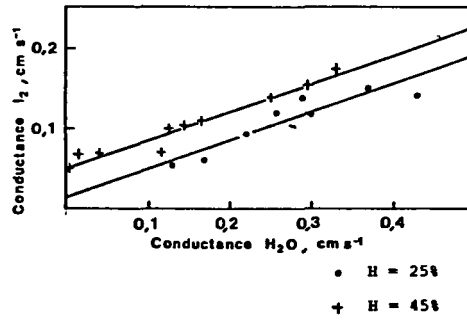

Fig. 1 : Transfert foliaire de l'iode par voie stomatique (cas d'une faible humidité relative). Les conductances foliaires vis-à-vis de l'iode et de la vapeur d'eau sont proportionnelles. L'ordonnée à l'origine, représentative du dépôt cuticulaire, croît avec l'humidité relative. Elle est voisine de zéro pour H = 25%.

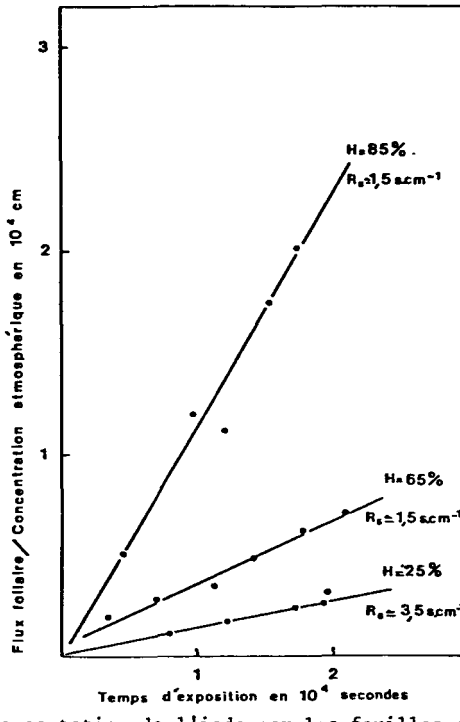

Fig. 2 : Cinétique de captation de l'iode par les feuilles dans différentes conditions d'humidité atmosphérique et de résistance stomatique (valeurs extrêmes et une valeur intermédiaire obtenues parmi 20 expériences). La vitesse de dépôt rapportée à l'unité de surface foliaire est donnée par la pente de la droite relative à l'essai considéré. La vitesse de dépôt rapportée à l'unité de surface de sol est obtenue en multipliant ce résultat par l'indice foliaire (surface foliaire/ surface sol).

Association EURATOM-CEA 099-76-1-PSAF

SOUS-CONTRAT N° : 028/099-PSTF
Centre de Développement des Etudes et Applications en Hygiène et Sécurité (CEDHYS), Avignon

Responsables scientifiques : J. CHALABREYSSE, R. COULON

ETUDE COMPARATIVE DE L'IMPACT SUR L'ENVIRONNEMENT DES INDUSTRIES CONVENTIONNELLES ET NUCLEAIRES : ASPECTS METHODOLOGIQUES

Les études qui ont été effectuées ont porté plus particulièrement sur l'analyse de l'impact des rejets atmosphériques d'une centrale au charbon en vue de la comparaison avec celui des rejets atmosphériques d'une centrale nucléaire et d'une centrale au fuel.

Au terme de l'annexe technique du contrat, les actions suivantes devaient être réalisées :

- un inventaire bibliographique de toutes les informations disponibles sur le sujet ;
- la mise au point des modèles prévisionnels permettant l'évaluation des conséquences des rejets à l'échelle régionale (dispersion atmosphérique, dépôt, dispersion dans les eaux, transferts dans les chaînes biologiques);
- l'étude in situ des paramètres d'environnement relatifs à la région étudiée (météorologie, hydrologie, démographie, pédologie, utilisation agricole du milieu...) ;
- l'étude des différents termes sources (nature des polluants, quantités rejetées, modalités de rejet, forme physico-chimique ;
- le choix des éléments polluants qui seront étudiés ;
- la mise en oeuvre d'un programme préliminaire de mesures radiologiques et chimiques portant sur les différents composants de l'environnement au voisinage des installations concernées.

La nature et l'importance des travaux effectués dans le cadre des thèmes indiqués ci-dessus vont être brièvement décrites :

1/ INVENTAIRE BIBLIOGRAPHIQUE
Les informations relatives aux centrales utilisant du combustible nucléaire ou fossile (charbon ou fuel) ont été rassemblées et analysées, notamment en ce qui concerne les connaissances relatives aux rejets de polluants, radioactifs ou non, et leurs transferts dans l'environnement.

2/ MISE AU POINT DES MODELES PREVISIONNELS
Les modèles qui seront utilisés sont, pour la plupart, décrits dans le document CEE n° N/3865/79 établi conjointement par le NRPB et le CEA. Quelques modifications leur ont été apportées pour tenir compte des caractéristiques d'émission et des caractéristiques d'environnement propres aux sites et à la région étudiée.

3/ ETUDE DES PARAMETRES D'ENVIRONNEMENT
Pour pouvoir évaluer la dispersion atmosphérique et le dépôt des différents polluants rejetés et quantifier leur transfert à la population on a entrepris de rassembler les principales caractéristiques régionales à savoir, les données météorologiques, les données économiques (productions agricoles) et les données démographiques (répartition géographique de la population).

4/ ETUDE DES TERMES SOURCES

L'étude bibliographique mentionnée ci-dessus a permis d'obtenir un certain nombre d'informations concernant les divers types d'installations. Par ailleurs, une campagne de mesure in situ des rejets atmosphériques d'une centrale au charbon a été effectuée. Cette centrale comporte 4 tranches (3 de 55 MW rejetant chacune par une cheminée de 65 m et une de 250 MW rejetant par une cheminée de 140 m de hauteur). Le charbon brûlé est extrait d'une mine située à proximité de la centrale. Les échantillons prélevés étaient :

- de la lignite en provenance de la mine,
- des cendres de foyer,
- des cendres volantes du dépoussiéreur,
- de l'air de la cheminée,
- des cendres humides des terrils.

Ces échantillons sont analysés en recherchant plus spécialement :

. sur la plan radioactif, U-238, Ra-226, Po-210 et Th-232 ;

. sur le plan non radioactif, des substances minérales (SO_X et NO_X (uniquement sur les prélèvements d'air), ainsi que Al, Cd, Cr, Co, Cu, Fe, Ni, Pb, V, Zn) ;

et des substances organiques (acides organiques, hydrocarbures polycycliques aromatiques).

Par ailleurs, une campagne de mesure similaire est entreprise dans le cas d'une centrale au fuel.

5/ CHOIX DES ELEMENTS ETUDIES

Ce choix, qui découle de l'inventaire bibliographique, se traduit par la recherche des polluants indiqués plus haut : en fonction des résultats une sélection pourra être opérée afin de ne conserver que les polluants les plus significatifs.

6/ MISE EN OEUVRE D'UN PROGRAMME PRELIMINAIRE DE MESURES DANS L'ENVIRONNEMENT

Des prélèvements ont été effectués dans l'environnement de la centrale au charbon qui a fait l'objet de la campagne de mesure des rejets atmosphériques.

Après étude de la rose des vents, deux points de mesure ont été déterminés. En ces deux points, des prélèvements de poussières atmosphériques portant sur quelques milliers de mètres cubes d'air ont été effectués. La granulométrie des poussières recueillies est déterminée ainsi que leurs concentrations en métaux (Al, Cd, Cr, Co, Cu, Fe, Ni, Pb, V, Zn). De plus, les prélèvements atmosphériques effectués font l'objet de mesure de concentrations en SO_X et NO_X.

Par ailleurs, des échantillons d'eau, de sol, de produits végétaux (salade, pommes de terre, carottes, raisin, épinards) et de produits animaux (crottes de lapin) font également l'objet d'analyse des mêmes substances radioactives et non radioactives.

Une synthèse de l'ensemble des données est en cours de réalisation : elle a pour objet d'une part de caler les évaluations prévisionnelles basées sur l'utilisation de modèles et les mesures réalisées in situ, d'autre part de mettre en évidence l'impact des rejets de la centrale pour une comparaison avec les impacts d'autres installations productrices d'énergie.

Association EURATOM-CEA 099-76-1-PSAG

SOUS-CONTRAT N° : 029-UK
Université d'Oxford

Responsables scientifiques : D. GRAY, S. PARISH

ANALYSE DE DONNEES EXPERIMENTALES RELATIVES AUX EFFETS DE L'INHALATION D'EMETTEURS ALPHA SUR LA CANCEROGENESE PULMONAIRE DU RAT

Certaines recherches expérimentales menées au sein du Service de Radiopathologie et de Toxicologie Expérimentales du CEA-DPr en vue d'améliorer la connaissance des déterminants du risque du cancer pulmonaire chez les mineurs d'uranium, comportent notamment l'exposition de lots d'animaux d'âge différent à des concentrations variables de radon, en association ou non avec diverses substances (tabac, benzoflavone, chimiothérapie anticancéreuse.

La présente étude a pour but de développer des méthodes appropriées pour procéder au traitement de ces données expérimentales et pour tenter de les modéliser. Les trois aspects suivants ont été abordés :

1. Etude de la relation dose-effet entre l'inhalation de radon et la prévalence de cancer du poumon chez le rat

Dans un premier temps, l'étude de la prévalence de cancer pulmonaire (représentée par tous les cas de cancers pulmonaires relevés dans la population étudiée), dans les lots de rats soumis à des expositions différentes, a conduit à préciser le mode de mortalité des animaux afin d'éviter tout biais dans la méthode de comparaison. A cet égard, il est apparu fondamental de connaître le délai séparant l'irradiation de la mort par tumeur, ce qui amène à distinguer les tumeurs létales qui sont diagnostiquées à l'occasion de la mort spontanée de l'animal et les tumeurs découvertes à l'occasion de la mort du rat par affection intercurrente ou au cours d'un programme de sacrifice systématique.

Les données semblent indiquer que le cancer du poumon détecté n'est pas létal habituellement et que l'excès de mortalité après forte exposition n'est pas dû à l'augmentation de l'incidence des cancers.

L'analyse de la prévalence tumorale en fonction de la dose d'irradiation permet de décrire une relation pratiquement linéaire si l'on exclut le lot d'animaux soumis à 3900 WLM, lot dans lequel on observe notamment l'espérance de vie la plus courte.

Par ailleurs, l'hypothèse d'une inhibition du développement ou de la détection des tumeurs du poumon par des lésions non néoplasiques du parenchyme pulmonaire est suggérée pour expliquer l'absence de relation dose-prévalence tumorale en cas de mortalité précoce.

2. Etude de l'influence de l'âge de l'animal lors de l'exposition

En première analyse, les résultats n'indiquent aucune relation entre la prévalence brute de cancer du poumon et l'âge au moment de l'exposition. Cependant, la distinction entre tumeurs létales et non létales montre que le risque d'apparition des tumeurs pulmonaires au bout d'un temps donné s'accroît avec l'âge de l'animal lors de l'exposition. Ce résultat concorde avec les observations faites chez les mineurs d'uranium.

3. Etude des effets combinés de l'exposition au radon et à d'autres agents

L'interprétation des observations réalisées sur l'homme oblige fréquemment à prendre en compte le phénomène de compétition de risque. Plusieurs cofacteurs ont été étudiés pour préciser cet aspect du problème :

3.1. Exposition au tabac

L'étude de différents lots de rats soumis à la fumée de cigarette avec ou sans inhalation conjointe de radon met en évidence une action synergique puissante, avec un excès considérable de cancer du poumon dans le groupe soumis aux deux produits.

3.2. Agents chimiothérapiques anticancéreux

L'étude de rats exposés à l'inhalation de radon (6-7000 WLM) et soumis parallèlement à différentes drogues anti-cancéreuses (Endoxan, Bléomycine, Méthotrexate ..) n'a pas permis de mettre en évidence une quelconque réduction de la prévalence de cancers pulmonaires dans les lots examinés.

3.3. Cérium 144 - Fer 59

L'expérience se propose de vérifier si l'induction des tumeurs par le cérium exerce un effet quelconque sur les cancers du poumon induits par le radon et si le Fer 59, utilisé en tant que traceur dans l'étude de la clairance pulmonaire, n'agit pas en synergie avec le radon. Les résultats de l'étude de 3 lots de rats n'ont pas permis de conclure.

3.4. SO_2

Le résultat des analyses conduites sur des lots d'animaux soumis à différents protocoles d'exposition au radon et à ce polluant atmosphérique montre que la survie la plus brève est observée en cas d'exposition au SO_2 préalable à celle du radon et que la survie la plus longue est celle des animaux uniquement exposés au radon. Cependant, la fréquence des cancers du poumon est identique dans tous les lots.

4. Elaboration de protocoles expérimentaux

L'ensemble des données recueillies dans le laboratoire au cours des dix dernières années a fait l'objet d'une étude critique en vue d'apporter d'éventuelles modifications aux futurs protocoles d'expériences. Les principales conclusions de ce travail soulignent

trois impératifs :

- nécessité d'une randomisation des rats dans chaque expérience

- nécessité d'une étude simultanée de différents niveaux de doses afin d'éviter le biais éventuel d'une modification dans la sensibilité de la souche de rats au cours du temps

- nécessité de porter une attention particulière à la puissance statistique de l'étude (rapport entre la taille des échantillons et la dimension de l'effet attendu).

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I976

ANGELETTI L., ANCELLIN L., BITTEL R.
Aspects pratiques du comportement du plutonium dans l'environnement
Radioprotection, volume 12, N° 1, I976

APSIMON H., GODDARD A.J.H. (Imperial College)
Modelling the atmospheric dispersal of radioactive pollutants beyond the first few hours of travel (7th International Technical Meeting on Air Pollution Modelling and its application
Sept 7-10, I976, Airlie, Virginia (USA)

GARNIER A.
Méthodologie d'évaluation des doses à la population, résultant des rejets radioactifs dans l'atmosphère
Rapport CEA-R-4781, I976

I977

ANGELETTI L., LEVI E.
Etude comparative des facteurs de transfert de l'eau, de l'iode et du strontium sur le ray-grass et le trèfle - Proposition d'un modèle d'évaluation des limites de la contamination foliaire par le dépôt humide
Rapport CEA-R-4860, I977

ANGELETTI L., LEVI E.
Etude du dépôt humide et de la rétention foliaire de l'iode et du strontium sur le ray-grass et le trèfle
Rapport CEA-R-4897, I977

APSIMON H., GODDARD A.J.H. (Imperial College)
Atmospheric dispersal of radioactive pollutants over long distances and the calculation of collective doses - 4ème Congrès International, IRPA, Paris, 24-30 Avril I977, P 403

GARNIER A.
Evaluation des doses collectives et comparaison des doses individuelles moyennes et maximales résultant des risques d'ingestion de lait originaire d'une région soumise à l'influence des rejets atmosphériques continus - 4ème Congrès International, IRPA, Paris, 24-30 Avril I977, P 151

MACCIA C., STEMMELEN E., PAGES P., MAITRE P.
Contamination par la chaîne alimentaire - Prévision des échanges agricoles et calcul des doses délivrées - Le cas du blé en France
Rapport CEA-R-4885, I977

MACCIA C. (EUR), MAITRE P., PAGES P., STEMMELEN E. (LSEES)
Analyse et prévision des échanges de produits agricoles entre différentes régions afin d'évaluer les contaminations par la chaîne alimentaire - 4ème Congrès International, IRPA, Paris, 24-30 Avril I977, P 426

I978

GARNIER A.
Comparaison de différentes méthodes de prévision de transfert atmosphérique (Séminaire sur la dispersion en milieu physique naturel, Cadarache, 13-17 Mars I978)

LAFUMA J.
Cancers pulmonaires induits par différents émetteurs alpha inhalés - Evaluation de l'influence de divers paramètres et comparaison avec les données obtenues chez les mineurs d'uranium - Communication au Colloque International sur les effets biologiques différés des rayonnements ionisants, AIEA, Vienne, 13-17 Mars I978, SM-224/I09, p 531

I979

ANGELETTI L.
Les transuraniens - Propriétés physico-chimiques et comportement dans l'environnement - Comportement dans les sols, les eaux et les sédiments - Transferts à la biomasse aquatique et aux végétaux
Rapport CEA-R-4987, I979

APSIMON H., WRIGLEY J. (Imperial College, London), LE GRAND J. and DESPRES A. (CEA, Fontenay-aux-Roses)
Application of the mesoscale diffusion models MESOS and TALD to accidental releases of radioactivity - Paper presented by H. BULTYNCK (SCK/CEN, Mol)
IOth International Technical Meeting on air pollution modelling and its application, Rome, 23-26 October I979

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Revue de quelques méthodes d'aide à la décision - Actes du Séminaire Européen sur les " Méthodes d'optimisation de la protection dans le domaine nucléaire, Luxembourg, 3-5 Octobre I979 (à paraître)

LOMBARD J., OUDIZ A.
Utilisation d'une analyse multicritère en vue de l'optimisation des rejets du cycle PWR - Actes du Séminaire Européen sur les " Méthodes d optimisation de la protection dans le domaine nucléaire ", Luxembourg, 3-5 Octobre I979 (à paraître)

LOMBARD J., OUDIZ A.
Une approche multicritère de la rationalisation des choix de radioprotection, Proceedings of a Seminar held in Vienna, 5-9 March I979 " Application of the doses limitations system for radiation protection - Practical implications", IAEA, Vienna, I979

OUDIZ A., UZZAN G.
Quelques options méthodologiques destinées à l'optimisation de la radioprotection - Proceedings of a Seminar held in Vienna, 5-9 March I979 " Application of the dose limitation system for radiation protection - Practical implications ", IAEA, Vienna, I979

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Programme d'études épidémiologiques de l'Association EURATOM/CEA-DPr : Problèmes posés par l'établissement d'un registre européen des travailleurs exposés aux radiations ionisantes, Congrès de l'IAEA, SM-237/32, Vienne, 26-30 Mars I979, sur " Les effets biologiques des radionucléides rejetés par les Industries Nucléaires ".

WRIGLEY J., APSIMON H., GODDARD A.J.H.
Meteorological data and the MESOS model for the long-range transport of atmospheric pollutant (W.M.O., Symposium on the long-range transport of polluants .., Sofia, 1-5 October I979

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ANGELETTI L.
Quelques remarques sur le devenir des transuraniens dans le milieu aqueux, Réunion Mixte AIEA/CEE, Ispra, Mars I980

ANGELETTI L.
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Séminaire Européen sur les rejets radioactifs et leur dispersion dans l'atmosphère après accident hypothétique de réacteur
Risø, 22-25 Avril I980

ALLWRIGHT S., McAULAY I. and COLGAN T.
A study of background radiation and cancer mortality in Ireland
Joint Meeting of the Society for Social Medicine, 2 Juillet I980, Leicester

BRENOT J., GARNIER A., HERBECQ J.M.
Radiocontamination de l'homme par la chaîne alimentaire - Le cas des produits de la mer en France en I976
Rapport CEA-R-5043, I980

GARNIER A., BRENOT J., OBINO A.M., HERBECQ J.
Modélisation des échanges de produits alimentaires en vue de l'évaluation des doses collectives - 5th International Congress of the IRPA, Jérusalem, March 9-14, (Vol. I, page 137), I980

GARNIER A., SAUVE A., MADELMONT C.
Représentation géographique de diverses données dans une grille européenne, 5th International Congress of the IRPA, Jérusalem, March 9-14, (Vol. I, page 307), I980

LOCHARD J., MACCIA C., PAGES P. (CEPN), BRENOT J., GARNIER A. (CEA)
L'effet de site sur l'impact des radionucléides rejetés dans le milieu marin - Colloque International sur l'impact des radionucléides rejetés dans le milieu marin, Vienne, Octobre I980

LOCHARD J., OUDIZ A.
Un modèle coût-bénéfice pour l'évaluation et la comparaison des programmes de prévention en santé publique
Communication orale présentée à la Conférence Internationale sur la Science des Systèmes dans le domaine de la Santé, Montréal, 14-17 Juillet I980

OUDIZ A.
Une approche multidimensionnelle de l'optimisation de la radioprotection - Revue " Radioprotection ", Vol. 15, N° 1, I980

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La détermination du risque radiologique acceptable : une approche basée sur la comparaison des coûts marginaux de protection dans diverses industries - 5th International Congress of the IRPA, Jérusalem, March 9-14, (Vol. I, page 215), I980

A PARAÎTRE EN I98I (sous presse)

BAILEY M.J., SLAZENBERG J.
A computer program for the location of adresses in Ireland, Irish Journal of Environmental Research and Development

GARNIER A., SAUVE A.
Etude géographique de la population européenne en vue de l'évaluation des conséquences radiologiques des rejets d'effluents radioactifs (rapport CEA-R)

LOMBARD J., OUDIZ A.
Analyse multicritère des choix de radioprotection - Approche méthodologique dans le cas des rejets radioactifs des installations nucléaires américaines
Rapport CEA-R-5086, I98I

LOMBARD J.
La rationalisation des choix de radioprotection : revue des principales méthodes envisageables - Rapport CEA-R

LOMBARD J., OUDIZ A.
L'approche par les fonctions d'utilité de la rationalisation des choix de protection - Rapport CEA-R

McAULAY I.R., COLGAN P.A.
Gamma-ray Background radiation measurement in Ireland
Journal of Health Physics, I980, 39, 5, 821-826

OBINO A.M., GARNIER A., BRENOT J.
Radiocontamination de l'homme par la chaîne alimentaire : le cas des produits laitiers dans la C.E.E. en I977
Rapport CEA-R

OUDIZ A., and aL, Editors
Optimization of Radiation Protection : Present Experience and Methods : Proceedings of the European Scientific Seminar held in Luxembourg, 3-5 October I979, Pergamon Press

OUDIZ A., LOMBARD J., FAGNANI F.
A multi-attribute approach to the rationalisation of radiological protection,Journal of Health Physics

UZZAN G., MAXIMILIEN R., SAUVE A.
Aspects méthodologiques du bilan de l'irradiation de la population, Second Special Symposium on Natural Radiation Environment, 19-23 Janvier I98I, Bombay

Contractor: International Commission on Radiological Protection.
Contract No: 180-76-1 BIOC.
Head of research team: Bo Lindell, Chairman, ICRP.
General subject of contract: Examination of the scientific problems related to radiation protection with a view to improving the planning and application of the results of research to this question.

Title of the project: Preparation of recommendations on radiation protection.
Head of project and scientific staff: Bo Lindell.

At the beginning of the term of this contract, ICRP was completing the review of its basic recommendations on radiation protection; this was published in 1977 as Recommendations of the International Commission on Radiation Protection (ICRP Publication 26).

Since its foundation in 1928 ICRP has maintained a continuing review of the biological bases of radiation protection, and in the past thirty years it has published five basic sets of recommendations. As will be shown, ICRP has initiated, within the last four years, a program intended to develop practical guidance on the policies recommended in ICRP Publication 26, and, at the same time, to continue studying the biological effects of irradiation so as to ensure a sound basis for radiation protection.

One of the most important features of ICRP Publication 26 is its emphasis on the need to keep all justified exposures from radiation as low as reasonably achievable, with dose limits as additional boundary constraints for the most highly exposed individuals. This emphasis contrasts with the former concept that the dose limits themselves could be used for purposes of planning and design. Instead, ICRP now recommends that the planning and design of installations involving exposure of people should take account of the recommendation that such exposures should be minimised to the lowest practicable level.

Because of this emphasis on keeping doses as low as practicable, ICRP has reviewed a number of its former publications in order to ensure that they conform to this basic principle. Plans were therefore made to prepare revised versions of the following reports:

ICRP Publication 7.	Principles of environmental monitoring related to the handling of radioactive materials.
ICRP Publication 12:	General principles of monitoring for radiation protection of workers.
ICRP Publication 13:	Radiation protection in schools for pupils up to the age of 18 years.
ICRP Publication 15/21:	Protection against ionizing radiation from external sources.
ICRP Publication 16:	Protection of the patient in x-ray diagnosis.
ICRP Publication 17:	Protection of the patient in radionuclide investigations.

It is expected that most of these reports will be completed in 1981.

In addition to the revised reports referred to above, ICRP initiated work on three urgent aspects of radiation protection.

- The important principle of keeping all justified doses as low as reasonably achievable has to be applied in a number of differing circumstances, and therefore ICRP decided to prepare a report giving advice on the practical application of this fundamental recommendation. This should be completed in 1981.

- Natural radiation constitutes the main exposure to man, and it varies greatly in different places. Some variations are caused by man-made activities, such as the construction of buildings. The extent to which the ICRP system of dose limitation should be applicable to natural background radiation is the subject of a special report that is currently being worked on.

- One particularly important aspect of exposure from natural background radiation is that of underground miners to radon and its daughter products. ICRP has recently recommended an appropriate limit for occupational exposure to radon and its daughter nuclides. It took as the basis for this limit the level of risk

corresponding to the present limit on effective dose equivalent of 50 mSv in a year. There are several ways of assessing the relationship between the inhaled amount of radon and its daughters and the level of risk. The dosimetric method used for most radioactive materials in ICRP Publication 30 and a similar method, slightly modified because of the special problems of the short-lived daughters of radon, have both been used. Epidemiological studies have provided a third method. There is a reasonably close agreement between the results of these methods, and ICRP recommends a limit which is at the low end of the dosimetric results and which is consistent with the epidemiological conclusions. These conclusions are not specific to radon because they relate to the consequences of exposure to the whole mining environment which includes some potentially hazardous non-radioactive agents. An ICRP report is being prepared for publication.

The recommended annual limit for intake by inhalation (the ALI) for radon-222 daughters, in terms of inhaled potential α energy, is 0.02 J in a year. The corresponding derived air concentration expressed in the practical units previously widely used is then 0.4 working levels.

As indicated above, ICRP regards as one of its main functions the responsibility of maintaining a continuing review of the fundamental bases of radiation protection. During the past four years ICRP has reviewed the very extensive epidemiological and radiobiological information that has become available and has concluded that the new information does not call for changes in the risk factors for stochastic effects or the dose-effect relationships for non-stochastic effects underlying the dose-equivalent limits recommended in ICRP Publication 26.

In ICRP Publication 26 ICRP concluded that a dose equivalent in the lens of the eye accumulated over a working lifetime of 15 Sv would not produce opacities that would interfere with vision. The ICRP's committee on radiation effects has reviewed the available human information and has concluded that, at this level of accumulated dose equivalent, some opacities might be produced which, while not in themselves detrimental to vision, might develop without further exposure to the point of causing deterioration of vision.

Although the combined effects of the present dose-equivalent limit for skin and the effective dose-equivalent limit make it very unlikely that dose equivalents in the lens would reach 15 Sv in a working lifetime, ICRP has decided to reduce its recommended dose-equivalent limit for the lens of the eye from 0.3 Sv in a year to 0.15 Sv in a year.

In 1976 ICRP had started to revise the values of the Annual Limits for Intakes (ALIs) of radionuclides by workers that had previously appeared in ICRP Publication 2 (1969). In 1978 it decided to extend this process to include all radionuclides with half-lives greater than 10 minutes. By 1980 ALIs had been published, in ICRP Publication 30, for over four hundred radionuclides of fifty-one elements; ALIs for a further forty-two elements are expected to be available in 1981.

The system of dose limitation now used by ICRP takes account of all body tissues that are irradiated following intake of the radioactive material, instead of only the critical organs as previously. The system ensures that the total risk from irradiation of any combination of organs does not exceed that from irradiation of the whole body at the recommended dose-equivalent limit. This summation of risks from individual organs can now be made on the basis of the much better knowledge of the sensitivity of each organ to radiation damage than was available 20 years ago. These improvements have in themselves caused only small changes in the values of ALI for individual radionuclides, but might require a reduction in the limits for some mixtures of radionuclides.

Much larger changes, however, have resulted from improved knowledge of the uptake and retention of radionuclides in body tissues, and of the radioactive decay schemes of some radionuclides. As a result of this new information, a few values of ALI now recommended in ICRP Publication 30 are substantially greater, and others substantially smaller, than those that can be derived from ICRP Publication 2.

PUBLICATIONS

ICRP Publication N° 24 / 1977
Radiation Protection in Uranium and other Mines

ICRP Publication N° 25 / 1977
The Handling, Storage, Use and Disposal of Unsealed Radionuclides in Hospitals and Medical Research Establishments

ICRP Publication N° 26 / 1977
Recommendations of the International Commission on Radiological Protection

ICRP Publication N° 27 / 1977
Problems Involved in Developing an Index of Harm

ICRP Publication N° 28 / 1978
Statement from the 1978 Stockholm Meeting of the International Commission on Radiological Protection

The Principles and General Procedures for Handling Emergency and Accidental Exposures of Workers

ICRP Publication N° 29
Radionuclide Release into the Environment : Assessment of Doses to Man

ICRP Publication N° 30, Part 1 / 1979
Limits for Intakes of Radionuclides by Workers

ICRP Publication N° 30, Supplement to Part 1 / 1979
Limits for Intakes of Radionuclides by Workers

ICRP Publication N° 31 / 1980
Biological Effects of Inhaled Radionuclides

ICRP Publication N° 30, Part 2 / 1980
Limits for Intakes of Radionuclides by Workers

IV.

LISTE DER VOR 1980 BEENDETEN VERTRÄGE

LIST OF CONTRACTS TERMINATED BEFORE 1980

LISTE DES CONTRATS TERMINES AVANT 1980

IV. CONTRACTS TERMINATED BEFORE 1980

Dosimetry

188-BIO UK	CEGB, Berkeley (Wheatley)*
169-BIO UK	NPL, Teddington (Lewis)**
246-BIO UK	Univ. Aberdeen (Mallard)***
097-PST UK	CEGB, Berkeley (Wheatley)*
107-PST D	KFA, Jülich (Heinzelmann)***
110-PST N	TNO, Arnhem (Julius)***

Radioactive contamination of the environment

186-BIO UK	AERE, Harwell (Chamberlain)**

Short-term effects of ionizing radiations

191-BIO EIR	Univ. Dublin (Mullins, Greally)***
213-BIO D	Univ. Giessen (Lohmann)***

Long-term effects of ionizing radiations

162-BIO I	CNEN, Bologna (Prodi)**

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* Final report in 1977

** Final report in 1978

*** Final report in 1979

V

KOORDINIERUNGSTÄTIGKEIT

COORDINATION

ACTIVITES DE COORDINATION

(1980)

V. COORDINATION

Study group meetings, symposia and conferences have proved to be a most effective means of coordination because they are naturally adapted to scientific work and easily accepted by scientists. These meetings, focusing on the evaluation of particular subject areas of the programme, are attended by research workers involved in the contract programme, as well as scientists from non-participating laboratories or organizations and scientific staff members of the Commission.

On the following pages the various meetings held in 1980 are listed :

A. Meetings of Study Groups, where scientists involved in the contract programme, independent experts and staff members of the Commission discuss specific subject areas of the programme.

B. Meetings organized or co-organized by the Commission.

C. Meetings of Experts, whose activities have the effects both of coordinating and stimulating efforts towards practical measures of radiation protection, in accordance with Chapter III of the Euratom Treaty.

A

MEETINGS OF

STUDY GROUPS IN 1980

Study Group "Calibration of Dosimeters used in Radiation Protection"

Luxembourg, 16-17 January 1980
17 Participants.

Principal subject :
Calibration problems of measuring instruments for use in radiation protection

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Study Group "Collection and Evaluation of Neutron Dosimetry Data"
(CENDOS Committee)

Brussels, 25 February 1980
5 participants from 3 countries and the Commission.

Principal subject :
Discussion of the state-of-the-art of projects and planning of future activities.

Study Group "Radiation Immunology"

Brussels, 3 March 1980.
6 participants from 2 countries and the Commission.

Principal subjects :
Orientation of future research concerning Radiation protection and radiobiological aspects in immunology.

Study Group "Radiation Protection in Nuclear Power Plants"

Luxembourg, 6 March 1980
5 participants

Principal subject :
Discussion on "Job-related" doses.

Study Group "Common goods containing radioactive substances"

Brussels, 25 March 1980
5 participants

Principal subject :
Radiation Protection related to consumer products containing radioactive substances.

Study Group "Radiation Protection dosimetry"

Luxembourg, 5 May 1980
14 participants

Principal subject :
Meeting of technical experts on radiation protection dosimetry.

Study Group "Calibration of Dosimeters used in Radiation Protection"

Luxembourg, 6-7 May 1980
17 participants

Principal subject :
Calibration problems of measuring instruments for use in radiation protection.

Study Group "Chemical structures, associated pollutants and behaviour of radionuclides"

La Baule, 6-8 May 1980
12 participants from 6 countries and the Commission.

Principal subjects :

- Behaviour of radionuclides and associated pollutants in different marine ecosystems (In situ studies);
- Biogeochemistry of trace elements in sediments;
- Results obtained in controlled conditions.

Study Group "Primary effects of ionizing radiation on nucleic acids"

Rotterdam, 27 and 28 August 1980
21 participants from 7 countries and the Commission.

Principal subject :
Future orientation of work; presentation of research programmes accepted for new contracts by the Commission. Initiation of collaboration.

Study Group "Dosimetry"

Rome, 3-4 November 1980
42 participants from 5 countries and the Commission.

Principal subject :
Organization of future research in dosimetry and exchange of information on the following subjects :

- Quantities and units
- Reference radiations
- Health physics dosimetry and instrumentation
- Microdosimetric studies and physical data
- RBE and epidemiology
- Intercomparison.

Study Group "Collection and Evaluation of Neutron Dosimetry Data"
(CENDOS Committee)

Rome, 5 November 1980
6 participants from 5 countries and the Commission.

Principal subjects :
Discussion of the state-of-the-art of projects and planning of future activities.

Study Group on "Behaviour of Technetium in the Environment"

Louvain-la-Neuve, 4-5 November 1980
20 participants from 7 countries and the Commission.

Principal subjects :
- Biochemical and toxicological aspects of Technetium;
- Technetium in marine environment;
- Technetium in dulcicol ecosystems;
- Technetium in terrestrial environment.

Study Group "Radiation Protection dosimetry"

Luxembourg, 10 November 1980
28 participants

Principal subject :
Meeting of technical experts on radiation protection dosimetry.

Study Group "Radiation risks resulting from medical uses of radiation"

Brussels, 26 November 1980
12 participants from 5 countries and the Commission.

Principle subject :
Harmonization of current and planned research within the framework of the Radiation Protection Programme 1980-1984.

Study Group "Epidemiological studies"

Brussels, 26 November 1980
14 participants from 3 countries and the Commission.

Principle subject :
Harmonization of current and planned research within the framework of the Radiation Protection Programme 1980-1984.

Study Group "Radiation risks resulting from nuclear activities, natural radiation and technologically enhanced levels of exposure"

Brussels, 27 November 1980
14 participants from 3 countries and the Commission.

Principle subject :
Harmonization of current and planned research within the framework of the Radiation Protection Programme 1980-1984.

Study Group " Radiation passport"

Luxembourg, 4 December 1980
20 participants.

Principal subject :
Discussions on problems concerning the radiation passport.

B

MEETINGS ORGANIZED OR CO-ORGANIZED BY THE
COMMISSION OF THE EUROPEAN COMMUNITIES IN 1980

Behaviour of transuranics in the aquatic environment and sediment-water exchanges ; techniques for identifying speciation

(JRC ; IAEA ; DG XII Biology - Radiation Protection)

Ispra, 24-28 March 1980
42 participants from 11 countries and the Commission.

Principal subjects :

- Methods for the determination of transuranic ;
- Chemical speciation at environmental levels ;
- Methodologies for studying the bioavailability of transuranics in aquatic organisms ;
- Chemical methods for the determination of metal fractions and applicability to transuranics on fresh estuarine and coastal sediments.

Seminar on Radioactive releases and their dispersion in the atmosphere following a hypothetical reactor accident

Risø (Denmark), 22-25 April 1980.
108 participants from 15 countries and the Commission.

Principal subjects :

- Characteristics of accidental releases ;
- Atmospheric phenomena influences ;
- Deposition ;
- Short range models ;
- Intermediate and mesoscale range models ;
- Experimental studies.

Seminar on chemical structures, associated pollutants and behaviour of radionuclides

La Baule (France), 6-8 May 1980.
12 participants from 6 countries and the Commission.

Principal subjects :

- Behaviour of radionuclides in areas under the influence of discharges of low-level liquid wastes from re-processing plants (Influence of local conditions ; re-processing practices...)
- Preliminary results of the Danish investigations at Thule ;
- Role of the non-radioactive pollutants associated with low-level wastes discharges.

15th Annual meeting of the European Society for Radiation Biology

Rotterdam, 25 to 28 August 1980
267 participants from 21 countries.

Principal subjects :
Modern trends in Radiation Biology have been presented and discussed by about 140 papers : the shape of the dose-effect curves in the low dose region; combined effects as well of high and low LET radiation as of drugs and radiations; extrapolation from one species to another; differences between stem cells and clonogenic cells; oxygen effect a.o.

7th international chromosome conference

Oxford, 26-30 August 1980
Approximately 300 participants from 20 countries.

Principal subjects :
Organization of chromosomes at the molecular level; chromosomes in the cell cycle and chromosomes in action; chromosomes in meiosis; population cytogenetics; chromosomes and speciation; chromosomes and malignant change; induced chromosome variation; human chromosomes (including origin of chromosome abnormalities in man, chromosome rearrangements, karyotype/phenotype correlations and genome mapping).

EULEP Symposium on Bone and Bone seeking radionuclides, physiology, dosimetry and effects.

Rotterdam, 29 August 1981
40 participants from 8 countries.

Xth international conference of yeast genetics and molecular biology

Louvain-la-Neuve, 8-12 September 1980
400 participants from 22 countries and the Commission.

Principal subjects :
Mitochondrial genetics; genetic engineering; regulation of metabolism; mutagenesis and repair; regulation of DNA transcription.

7th Symposium on Microdosimetry

Oxford, 8-12 September 1980
200 participants from 23 countries, international organizations and the Commission.

Principal subject:
Consequences of the microdistribution of absorbed energy from ionizing radiations for radiation protection:

- physical principles of radiation interaction and energy deposition, spatial and temporal distributions of radiation interaction processes, experimental and mathematical determination of energy deposition spectra,
- problems of radiation quality and its distribution within phantoms and the human body, relations between microdosimetric descriptions of radiation quality of mixed radiations and the mean quality factor,
- dependence of biomolecular and radio-chemical effects and kinetics on radiation quality and their explanation in terms of primary energy deposition processes,
- dependence of biological effects on external radiation and incorporated radionuclides and their interpretation in terms of microdosimetry, influence of local distribution and nature of sensitive targets,
- analysis and interpretation of dose-effect ralationships including epidemiological data, in terms of radiation effect mechanisms and their relevance to radiological protection,
- discussion of models of radiation mechanisms with particular regard to the influence of radiation quality and biological repair processes on dose effect relationships.

Seminar on the European Radiation Protection Dosimeter Intercomparison Programme

Grenoble, 6-8 October 1980
35 participants from 8 countries and the Commission.

Principal subject:
Discussion of results of beta-dosimeter intercomparisons carried out in 1979/1980 and of the problems encountered in beta-dose assessment.

European Seminar on Radiation Protection Quantities for External Exposure

Braunschweig, 13-15 October 1980
55 participants from 18 countries, international organizations and the Commission.

Principal subject:
Exchange of information and ideas to clarify the usefulness of currently discussed concepts for radiation protection quantities:

- Quantities used for defining basic and derived radiation protection limits (their purposes and their interrelationships).
- Concepts of operational quantities (purposes, theoretical considerations, calculated and measured data).
- Relationships between operational quantities and quantities used for specifying primary protection limits (i.e. effective dose equivalent, dose equivalent for specified tissues, mean whole body dose equivalent).
- Measurement and calibration problems associated with operational quantities.
- Purpose and function of operational quantities as seen from the demands of practical radiation protection.

C

MEETINGS OF EXPERTS IN 1980

Bruxelles - 7.3.1980 - Group of experts, art. 37 Euratom Treaty
15 participants
Subject: Reference accidents

Bruxelles - 26.3.1980 - Group of experts, art. 37 Euratom Treaty
35 participants
Subject: Power Stations - Recommendations

Bruxelles - 14.4.1980 - Working group
15 participants
Subject: Recommendations - art. 37 Euratom Treaty

Luxembourg - 12-13.5.1980 - Working group
26 participants
Subject: Basic Safety Standards

Bruxelles - 19.5.1980 - Working group
15 participants
Subject: Reference accidents - art. 37
Euratom Treaty

Rome - 10.6.1980 - Working group
15 participants
Subject: Meteo (art. 37 Euratom Treaty)

Bruxelles - 13.10.1980 - Working group
20 participants
Subject: Intervention levels in the event of
nuclear accidents

Bruxelles - 4-5.11.1980 - Working group
26 participants
Subject: Basic Safety Standards - art. 31
Euratom Treaty

Bruxelles - 18-19.12.1980 - Working group of experts
15 participants
Subject: Meteo (art. 37 Euratom Treaty)

VI

AUSWAHL EINIGER AUF VERANLASSUNG DER KOMMISSION ERSCHIENENER VERÖFFENTLICHUNGEN

SELECTION OF PUBLICATIONS ISSUED ON THE INITIATIVE OF THE COMMISSION

CHOIX DE PUBLICATIONS EDITEES A L'INITIATIVE DE LA COMMISSION

(1976-1980)

VI. PUBLICATIONS 1976-1980

The scientific research results of the Community's Radiation Protection Programme are presented in articles published in scientific journals. References to these are given in the corresponding project reports. In certain cases the Commission initiated surveys of detailed results of specific activities in the field of radiation protection and published them as monographs proceedings and radiological protection data as announced on the following pages :

A. Monographs and Proceedings
B. Radiological Protection Data
C. Other publications.

A.

MONOGRAPHS AND PROCEEDINGS

- Problems posed by the growing use of consumer goods containing radioactive substances

Proceedings of the seminar organized by the Commission of the European Communities (Health and Safety Directorate) in Luxembourg on 13/14 November 1975

The most important subjects treated during the sessions were the following :

- the practical application of the principles laid down in Article 12 of the amended Euratom Basic Safety Standards (population dose) ;
- the technical, economic and health criteria to be observed when authorizing the marketing of consumer goods containing radioactive substances ;
- special cases (plasters, ceramics, paints, electrical and electronic equipment containing radioactive substances ;
- state of national legislation in relation to the stipulations of articles 4 and 5 of the revised Basic Safety Standards (systems of reporting and prior authorization) ;
- the radiological protection methods to be applied when goods are in use and when they are discarded.

The last session - a round table discussion - was concerned with informing the public.

It emerges from the conclusions of this seminar that only action at Community level in this particular field of the health protection of the general public against risks from ionizing radiations can guarantee for useful and valid solutions.

Eur Report 5601 d/e/f, 1976, 161 pages
To be ordered through :

Office for Official Publications of the European Communities
Boîte Postale 1003 - Luxembourg

FB 450,- DK 71,- DM 30,50 FF 54,- Lit 8.150,- Fl 31,10 £ 5,50 $ 12,90

- Basic Physical data for Neutron Dosimetry, edited by J.J. BROERSE

Based on the results of a workshop on Basic Physical Data for Neutron Dosimetry held in Rijswijk (The Netherlands) on 19-21 May 1976, this monograph reviews the current status in neutron dosimetry and the agreements that were reached on the use of some common basic physical parameters. As appendices are joint tables of kerma factors and a draft of a protocol for neutron dosimetry for radiobiological and medical applications.

Main topics treated :

- Source and field characteristics
- Cross sections and mass energy transfer coefficients ; measurements and calculations
- Detector response, measurements and calculations
- Dose distributions in phantoms for a limited set of conditions
- Standardisation, calibration and intercomparison.

Eur Report 5629e, 1976, 323 pages, to be ordered through :

Office for Official Publications of the European Communities
Boîte postale 1003 - Luxembourg

FB 900,- DK 142,- DM 61,- FF 108,- Lit 16.300 Fl 62,20
£ 11,- $ 25,80

- Radioactivité des eaux de la Meuse

Compte-rendu du séminaire organisé par la Commission des Communautés européennes à Wepion (Belgique) les 24 et 25 avril 1975.

Le rapport établi sur base de données fournies par les services compétents des pays riverains de la Meuse, donne un aperçu des conditions radiologiques actuelles dans le bassin du fleuve; il montre comment la situation pourrait évoluer sur ce point et quels problèmes en résulteraient. Les questions touchant les Pays-Bas, qui de tous les riverains ont le plus à redouter une pollution des eaux de fleuve, ont été traitées de façon plus détaillée.

Internal Doc. nr V-3559/1/75, f, d, n, 1976, 60 pages,
Available at : Commission of the European Communities
Directorate General "Employment and Social Affairs"
Health and Safety Directorate
Bâtiment Jean Monnet, A-2/078
Luxembourg - Kirchberg

- Bone marrow transplantation and other treatment after radiation injury,
H. BALNER

This monograph deals mainly with current concepts about bone marrow transplantation as therapy for serious radiation injury. Such injury can be classified according to the following broadly defined dose ranges :

1) the supralethal range, leading mainly to the cerebral and intestinal syndromes;

2) the potentially lethal or therapeutic range which causes the bone marrow syndrome, and

3) the sublethal range which rarely leads to injury requiring therapy.

The bone marrow syndrome of man and animals is discussed in detail. The optimal therapy for this syndrome is bone marrow transplantation in conjunction with conventional supportive treatment. The principal complications of such therapy are Graft versus Host Disease and a slow recovery of the recipient's immune system. The review contains a number of guidelines for the handling of serious radiation accidents.

Co-ordinated research activities in a number of contracts with the European Communities and of other institutions have led to considerable progress in this field of bone marrow transplantation.

Of interest to : Authorities responsible for radiation protection, scientist working in radiation protection, health physics, radiation biology and radioimmunology, and medical doctors.

1977, 83 pages,
To be ordered through your bookseller, or
Martinus NIJHOFF Medical Division
P.O.B. 442 THE HAGUE/THE NETHERLANDS.

- First European Symposium on Rad-Equivalence

Proceedings of the Seminar on Radiobiology-Radiation Protection - Orsay (France) 24/26 May 1976

edited by R. CHANET

The principles and concepts used in radiation protection are presented as models providing solutions to the numerous problems raised by the monitoring of human and environmental contamination by chemical pollutants. One relatively recent hypothesis is the "rad-equivalent" and the objective discussions arranged by the Commission and Professor LATARJET centred on the possible uses of this concept, the attraction of which lies in its simplicity and practical utility.

The discussions were focussed in particular on the study of mutagens present in the environment, to which a concept of this kind could, in the opinion of its apologists, be applied most successfully.

Eur Report 5725 e, 1977, 265 pages ; to be ordered through :

Office for Official Publications of the European Communities
Boîte Postale 1003 - Luxembourg

FB 430,- DK 70,50 DM 28,- FF 58,- Lit. 10.200 Fl 29,20 £ 6,85 $ 11,80

- First Information Seminar on the European Personal Dosimeter Intercomparison Programme (Photon-Intercomparisons 1974/75)

21-23 June 1976 in Berlin

This seminar was attended by 70 experts from Member State research institutes and dose measurement stations with which the Commission has carried out comparative measurements in personal dosimetry for several years in an attempt to improve the accuracy of personal dosimeters used in practical radiation protection work. This work touches therefore directly on the important aspect of physical radiation protection surveillance as laid down in the recommendations and basic safety standards for the health protection of workers and the general public against the dangers of ionizing radiation.

The main purpose of this seminar was to make a final critical review of the results of the personal dosimeter comparison programmes carried out in Europe under the auspices of the European Commission in recent years - both from the point of view of the personal dosimetry processing centres and of the institutes which had produced the requisite irradiation for the comparisons - in order to consider the matching results obtained from the comparisons and the difficulties encountered.

Internal Doc. nr 1623/77, 1977, 154 pages,

Available at : Commission of the European Communities
Directorate General "Employment and Social Affairs"
Health and Safety Directorate
Bâtiment Jean Monnet
Luxembourg - Kirchberg

Information and Training on Radiation Protection for Trade Union Representatives from the nine Member States of the EC.

Papers presented at the first and second seminars on 7/8 October 1975 and 16/17 November 1976.

On 7/8 October 1975 and 16/17 November 1976 the Directorate-General for Employment and Social Affairs (Health and Safety Directorate) held the first and second information and training seminars for trade union representatives from the nine Community countries on aspects of worker protection in the nuclear energy industry.

The Commission's aim in arranging these seminars is to inform the representatives of the European Trade Union Congress of current problems and trends in radiation protection and to promote frank exchanges with union representatives, to take up problems and proposals emerging from everyday working life, and hence to set up a constructive dialogue which will assist union representatives and the Health and Safety Directorate in achieving optimum radiation protection for workers.

The programme for the first seminar comprised the following topics:

- Community powers and responsibilities in the field of radiological protection.
- Physical and medical surveillance in normal and accidental conditions.
- Protection of workers occasionally exposed to ionizing radiation.
- Occupational irradiation recorded in the Member States.
- Delayed effects of low radiation doses.
- Special problems caused by plutonium 239.
- Problems caused by radioactive waste.

The second seminar covered the following problems:

- Information for workers on the hazards of the nuclear industry and on radiological protection.
- The use of the radiation logbook in Germany.
- The role of radiological protection units.
- Problems of radiological protection with regard to the maintenance, repair and decontamination of nuclear power stations.
- Compensation for occupational diseases caused by ionizing radiation (Community principles, legislation in the Member States).
- Radiological protection of female staff (risks, scientific and legal aspects).

This publication contains reports presented at the 1975 and 1976 seminars. Although it is addressed basically to the participants at these two meetings, the document also contains useful information for all persons concerned with questions and information relating to radiation protection.

Internal document No 1957/77 DE/FR/EN

Available at : CEC
Directorate General "Employment and Social Affairs", Health and Safety Directorate,
V - F - 3
Bâtiment Jean Monnet A2/103
Luxembourg-Kirchberg

- Effects of ionizing radiation on DNA ; Physical, Chemical and Biological Aspects

A.J. BERTINCHAMPS (Co-ordinating editor)
J. HÜTTERMANN (Editor on Physical aspects)
W. KÖHNLEIN (Editor on Biological aspects)
R. TEOULE (Editor on Chemical aspects)

This book results from a common effort of the members of the European study group on "Primary effects of radiation on nucleic acids". It contains the state of knowledge in this field, written in close collaboration by 27 authors. For the first time the three essential approaches to research on effects of ionizing radiation on DNA and its constituents have been described together in one book, thus providing a handy tool for obtaining an overall view of the various aspects of the fundamental problems involved.

The first section (Physical aspects) gives a general background of the main physical - chemical properties of nucleic acids and emphasizes those which are relevant to radiation research. It deals mainly with the structure, yields and various properties of the free radicals induced by radiation in DNA in the solid state.

The second section (Chemical aspects) gives a survey of the primary processes as well as the final products in the radiolysis of aqueous solutions of DNA and its components.

The effects of radiation on DNA and its functions in the living state constitutes the essential of the third section (Biological aspects), detailed consideration being given to replication, transcription, repair, transformation, transfection, the methods of testing them, and the material in which it is usually or the most easily observed.

Of interest to : Radiation physicists, chemists and biologists, to graduate students in biochemistry, molecular biophysics and biology, and to specialists in fundamental radiation protection.

1978, 420 pages, 85 figures

To be ordered through your bookseller, or
Springer-Verlag Berlin, Heidelberger Platz 3, D-1 Berlin 33, or
Springer-Verlag New York Inc., 175 Fifth Avenue, New York, NY 10010, USA.

Biological Effects of ^{224}Ra, Benefit and Risk of Therapeutic Application

Proceedings of the Second Symposium at Neuherberg/München,
September 20-21, 1976
edited by W.A. MÜLLER and H.G. EBERT

The Second Symposium on the Biological Effects of ^{224}Ra, held at Neuherberg, was focussed on two topical aspects of radiation protection. One aspect was the long-term effects of high-LET ionizing radiations on man and the quantitative data involved in risk assessment at low doses. The evaluation of epidemiological studies and experimental research was discussed in order to provide facts and figures contributing to an objective assessment of the radiation hazard from incorporated radionuclides.

The other aspect was that of radiation protection in medicine. In the case of ^{224}Ra treatment of ankylosing spondylitis the questions of benefit and risk of this therapeutic use of ionizing radiations were discussed, the aim being to achieve the therapeutic effect while reducing radiation exposure - and therefore the hazard - to a minimum.

The proceedings contain the complete texts of 23 papers as well as the final round table discussions.

1978, 236 pages, to be ordered through your bookseller or
Martinus NIJHOFF Medical Division, P.O.B. 442, The Hague/THE NETHERLANDS

Investigations into the emission of Carbon-14 compounds from nuclear facilities

J. SCHWIBACH, H. RIEDEL. J. BRETSCHNEIDER

This report deals with the emission of carbon-14 from nuclear facilities (nuclear power plants and reprocessing facilities) and the resulting radiation exposure. A preliminary survey is made of studies concerning the calculation and assessment of the rates of formation and release of C-14 from reactors and reprocessing facilities and previous measurements of emission rates from such facilities are summarized. The methods adopted for sampling and measuring C-14 compounds in air are described, and the results of more than 700 individual measurements for light-water cooled reactors are reported. The results of calculations of the radiation exposure of the population are also discussed.

CEC November 1978 - Doc. V-3062/78-EN

Available at : CEC
Directorate General "Employment and Social Affairs", Health and Safety Directorate,
V - F - 3
Bâtiment Jean Monnet A2/103
Luxembourg-Kirchberg

- Third Symposium on Neutron Dosimetry in Biology and Medicine
23 to 27 May 1977

edited by G. BURGER and H.G. EBERT

The growing importance of nuclear energy and the applications of neutron sources in research, medicine and industry imply considerable efforts in radiobiological research and increased emphasis on the assessment of radiation hazards to man. Since the second Symposium on Neutron Dosimetry in Biology and Medicine was held in 1974, interesting results of research related to the physical aspects of neutron dosimetry have been achieved.

In particular, the discussion of relevant theoretical and experimental aspects of neutron dosimetry in mixed radiation fields is a prerequisite for the worldwide comparison of radiobiological and clinical results. Therefore the following topics have been embraced :

- Physical basis of neutron interaction and energy deposition ;
- Radiation quality and radiobiological implications ;
- Calculations of radiation transport ;
- Neutron sources, irradiation facilities and collimators ;
- Neutron spectrometry ;
- Neutron and mixed field dosimetry, methods and instrumentation, free-air and phantom measurements ;
- Calibration, standardization and intercomparison.

Eur Report 5848 d/e/f, 1978, 950 pages, in press,
to be ordered through :

Office for Official Publications of the European Communities
Boîte Postale 1003 - Luxembourg

Results of Environmental Radioactivity Measurements in the Member States of the European Community for Air - Deposition - Water - Milk, 1975-1976, Luxembourg 1978.

The present document is the sixteenth report published by the Health and Safety Directorate of the Commission of the European Communities concerning ambient radioactivity. It was drawn up using the data collected by the stations in charge of the surveillance of environmental radioactivity in the Member States. The results are extracts from the data sent to the Commission in application of Article 36 of the Treaty of Rome instituting the European Atomic Energy Community.

This is the second document which includes data from the enlarged Community viz. Belgium, the Federal Republic of Germany, France, Italy, Luxembourg, the Netherlands, plus Denmark, Ireland and the United Kingdom, who joined the Community of 1 January 1973.

The results presented in this report deal with radioactive contamination of the air, precipitation and fallout, surface water and milk during 1975 and 1976.

EUR Report 5944 DA/DE/EN/FR/IT/NL
1978 - 328 pages
to be ordered through
Office for Official Publications in the European Communities
Boîte Postale N° 1003
Luxembourg

BFR: 1.150,- - DKR: 203,- - DM : 74,- - FF : 167,- - LIT : 31.000,-
HFL: 79,- - UKL: 19,- - USD: 36,-

A European Neutron Dosimetry Intercomparison Project (ENDIP)
Results and Evaluation

edited by J.J. BROERSE, G. BURGER and M. COPPOLA

A total of twenty groups from nine countries participated in sessions of the European Neutron Dosimetry Intercomparison Project (ENDIP) which were held during 1975 at GSF, Munich-Neuherberg and TNO, Rijswijk. The data of all participants are collected, the analysis and evaluation of the results are given in the present report. Specific chapters deal with the experimental arrangements and monitoring results at GSF and TNO, characteristics of the dosimetry systems employed by the participating groups and the basic physical data and correction factors employed for the determination of kerma and absorbed dose. Recommendations for future studies on neutron dosimetry for biological and medical applications are given at the end of the report.

EUR Report 6004 EN, 1978, 175 pages, to be ordered through:

Office for Official Publications of the European Communities
Boîte Postale 1003, Luxembourg

Price: BFR 360,- DKR 63,50 DM 23,- FF 52,20 LIT 9.700,-
HFL 24,80 UKL 6,-- USD 11.30

Sixth Symposium on Microdosimetry

Brussels, 22-26 May 1978

edited by J. BOOZ and H.G. EBERT

The proceedings contain on 1261 pages 93 invited and contributed papers and 10 reports of poster sessions and summaries of discussions.

The emphasis of the Symposium was put on the application of microdosimetry in the fields of radiation protection, of radiobiology and -chemistry, and of radiotherapy and -diagnostics. The following main topics are discussed:

- biological and chemical principles of the radiation effects from external radiation and incorporated radionuclides, interpreted with the aid of microdosimetry;
- physical principles of radiation interaction and energy deposition;
- spatial and temporal distribution of radiation interaction processes in organs, cells and their substructures and biomacromolecules;
- analysis and interpretation of dose/effect relationships; models of radiation effect mechanisms; problems of radiation quality and quality factors.

EUR Report 6064 DE-EN-FR, 1978, 1261 pages, to be ordered through:

Harwood Academic Publishers Ltd.
Chansitor House, 37/38 Chancery Lande
London WC2A 7EL / U.K.

Intermediate Energy Neutron Production; A Survey of Existing Techniques; A proposed Source and its Applications

Final report of contract nr 135-74-7 BIO UK
Central Electricity Generating Board, Berkeley Nuclear Laboratories, England

A.J. MILL and J.R. HARVEY

A wide ranging study of possible techniques for producing intermediate energy neutrons has been made and the use of neutron filters in the beam tube of a high flux reactor has been identified as the most promising approach for general application. The technique is already used in a few reactor centres where neutron beams of energy 2 eV, 24 keV, 120 keV are produced with filters of scandium, iron and silicon respectively, placed in a neutron beam tube. It has been shown that it is possible in principle to extend the range of energies down to 50 eV by the use of single isotope filters. The lower energy neutron beams (below 2 keV) would be unique. They could be used, not only for calibrating a range of neutron-sensitive instruments, but also for studies including fundamental radio-biology, basic nuclear physics and for radiotherapy of brain tumours. A notional facility has been designed which could be located at a high flux reactor, of which seven in Europe are particularly appropriate. The cost of the isotopes would be about ₤1M, a cost comparable with conventional neutron-generating facilities.

EUR report 6107 EN, 1978, 88 pages, to be ordered through:

C.E.G.B. - Berkeley Nuclear Laboratories
Gloucestershire, U.K.

or

Office for Official Publications of the European Communities
Boîte Postale 1003, Luxembourg

Radiation induced chromosomal aberrations

Proceedings of the contractants meeting organized by the Commission of the European Communities in Brussels, 28-29 November, 1978.

These proceedings include the text of all presentations made by the contractants on the assessment of radio-induced non-disjunction and the understanding of the mechanisms involved :

Published by ELSEVIER, North-Holland, Biomedical Press, in 1979, as a special issue of Mutation Research, 61, 1, pp. 1-119, D. de Nettancourt and K. Sankaranarayanan editions.

Methodology for evaluating the radiological consequences of radioactive effluents released in normal operations - July 1979

A report prepared under contract for the Commission of the European Communities within its Research and Development Programme on "Plutonium Recycling in Light Water Reactors"

The aim of this report is to present a methodology for the evaluation of health detriment to the population of the countries of the European Community, from discharges within the community of liquid and gaseous radioactive effluents. This methodology is based on a series of sequential models describing the transfer of radionuclides through the different sectors of the environment, the pathways of exposure of man and the consequential health detriment. The methodology could be applied to evaluate individual exposure, but since health detriment is of interest here,the emphasis in the study has been in the estimation of collective dose and consequent numbers of health effects. The models chosen are of sufficiently general character that the corresponding methodology can find wide application for the evaluation of the radiological consequences of discharges of effluents.

CEC July 1979 - Doc. V/3865/79-EN-FR

Available at : CEC
Directorate General "Employment and Social Affairs", Health and Safety Directorate,
V - F - 3
Bâtiment Jean Monnet A2/103
Luxembourg-Kirchberg

Radiation-induced non-disjunction

Proceeding of a workshop organized by the Commission on October 28-29, 1979 in Brussels, which reviews the achievements and the current efforts of the contractants of the Commission who are studying radiation-induced non-disjunction in man and in experimental species. They provide up-to-date information on :

- the occurence of aneuploidy in man and in the progeny of humans with radiation history ;
- dose-response relationships ;
- the mechanisms which are suspected to lead to the manifestation of the trisomic condition.

The research efforts presented by the contractants involve the use of several different test systems developped in micro-organisms (Aspergillus and yeast), insects (Drosophila), plants (Petunia), and mammals (Mouse, Microtus) as well as direct observations on human material. Ten communications from Belgian, Dutch, British, Danish, German, and French scientists were presented which are reproduced in extenso in the proceedings. (Special issue of Mutation Research, Vol. 61, n° 1, June 1979 (pp. 119), Editors D. de Nettancourt and K. Sankaranayanan, published by Elsevier/North Holland).

To be ordered at :
Elsevier/North Holland
Biomedical Press
P.O.Box 211
NL - 1000 AE Amsterdam
Netherlands

Radioprotection - no 16 - 1979

Proceedings of the Seminars held in Luxembourg, 10-11 October 1977 and 12-13 October 1978 on :

Information and training on radiation protection for trade union representatives from the nine Member States of the European Communities

The following detailed subjects were dealt with at the third Radiation Protection Seminar:

- The independance of radiation protection units under the terms of Article 35 of the 1976 EURATOM Basic Safety Standards;
- Training in and information on radiation protection;
- Analysis of the main innovations emerging from ICRP 26;
- The protection of occasionally exposed workers.

The fourth Radiation Protection Seminar concentrated on the following subjects:

- The concept of optimization in radiological protection;
- Future progress in the use of dosimetry;
- Tasks and results of the United Nations scientific committee on the effects of atomic radiation.

Moreover, a first exchange of views took place at the fourth Radiation Protection Seminar on an enquiry carried out by the European Confederation of Trade Unions into radiation protection practice.

EUR 6264 DE/EN/FR - 1979
Office for Official Publications in the European Communities
B.P. 1003 - Luxembourg

Price: 320 BFR - 57.50 DKR - 20 DM - 46 FF - 8 900 LIT - 22 HFL - 5 UKL - 5,30 IRL - 10.50 USD

A Small Scale Neutron Dosimetry Intercomparison

edited by J.J. BROERSE, J. ZOETELIEF, G. BURGER, H. SCHRAUBE, A. RECOURT

During the past six years two extended neutron dosimetry intercomparisons have been performed, the International Neutron Dosimetry Intercomparison (INDI) under the sponsorship of the ICRU (1978) and the European Neutron Dosimetry Intercomparison Project (ENDIP) under the sponsorship of the Commission of the European Communities (ENDIP, 1978). The conclusions of both intercomparison programs are that there is a strong need for a common agreement upon the application of basic physical parameters and for standardization of experimental techniques applied for the determination of absorbed dose. In order to accomplish consistency in neutron dosimetry results obtained at different institutes, a small scale intercomparison was extended to analyse the existing discrepancies, to learn about detector characteristics and to decide on the appropriateness of different types of ionization chambers to be used as reference dosimeters.

EUR Report 6567 EN, 1979, 38 pages, to be ordered through:

J.J. BROERSE, Radiobiological Institute TNO
P.O.Box 5815, NL-2280 HV RIJSWIJK / THE NETHERLANDS

Chromosomal aspects of male sterility in mammals

Summary and synthesis, together with the list of individual contributions, of a workshop organized by P. de Boer (Department of Genetics, Agricultural University, Wageningen, the Netherlands) and A. G. Searle (M.R.C. Radiobiology Unit, Harwell, U.K.) with partial financial support of the Commission. The workshop was held at the M.R.C. Radiobiology Unit, Harwell on 24-26 October 1979, with two major aims : 1) to discover the similarities and differences between the male-sterile conditions associated with various forms of chromosome anomaly in mammals (mainly the mouse) and 2) to try and integrate the phenotypic pecularities of these syndromes with the basic cytological and physiological aspects pf spermatogenesis in general and male meiosis in particular.

The summary and synthesis of the workshop have been published under the heading :

"Workshop on chromosomal aspects of male sterility in mammals"

in : J. Reprod. Fert. (1980) 60, 257-265

Radiation repair in yeast

Proceedings of a workshop organized by the Commission on September 10, 1980 which reviews the state of knowledge on radiation repair in yeast. A summary of the present situation is provided on :

- the detection and quantification of lesions ;
- sensitivity mutations and repair models ;
- repair functions ;
- inducibility ;
- repair activity at different stages of the cell cycle ;
- the need to explore in details the biochemistry of repair.

The proceedings contain the abstracts of papers contributed for the workshop.

Copies can be obtained from :
Prof. A. Goffeau
U.C.L.
Enzymologie
Place Croix du Sud, 1
Boîte 8
B - 1348 Louvain-la-Neuve
Belgium

Seminar on Radioactive releases and their dispersion in the atmosphere following a hypothetical reactor accident

Proceedings of the Seminar held in Risø (Denmark), 22-25 April 1980.

The aim of the seminar, jointly organized by the Health and Safety Directorate (DG V) and the Biology, Radiation Protection Programme (DG XII) of the Commission was to provide a review of the currently available data on atmospheric phenomena and parameters involved in the atmospheric dispersion of gaseous effluents, over distances up to and including the mesoscale, and of current dispersion modelling techniques.

The summaries of the sessions were presented by the Chairmen at the end of the Seminar (Panel session).

The set of release and dispersion data will be considered in the framework of a second seminar dealing with the transfer and analysis of the risks of atmospheric releases to man and its environment.

The proceedings contain the papers contributed for the Seminar.

Copies can be obtained from :

Commission of the European Communities
D.G. XII - Biology, Radiation Protection and Medical Research
Rue de la Loi, 200
B-1049 BRUSSELS

Irradiation and thyroid disease : dosimetric, clinical and carcinogenic aspects.

J.E. Dumont, J.F. Malone, A.J. Van Herle.

Thyroid cancer caused by irradiation is an important public health problem because of the many possible causes of external and internal exposure: accidental, diagnostic and therapeutic. Three aspects are considered. Data in the literature are analysed to define the quantitative relation in man between irradiation and thyroid disease : thyroid insufficiencey and neoplasia. The clinical characteristics of nodules and cancers of this origin, the prognosis and the therapeutic attitude are discussed. Next the radiobiology and the dosimetry of the thyroid are described and the necessary relation between the study of both is emphasized. The relative radioresistance of the gland is demonstrated. The characteristics of thyroid growth regulations is then discussed in relation with current concepts of carcinogenesis. The possible application of experimental in vivo and in vitro data and these current concepts to the case of irradiation-induced thyroid cancer is considered. New lines of research are suggested.

EUR 6713, 1980, 254 p.
Medicine series

to be ordered at
Office for Official Publications of the European Communities
Boîte Postale 1003
GD-Luxembourg.

Price : BFR 800 DKR 153,60 DM 49,20 FF 115,60 IRL 13,40
LIT 22.800 HFL 54,20 UKL 12.10 USD 27.60

Ion chambers for neutron dosimetry

edited by J.J. BROERSE

This monograph reviews the current status of ion chambers and their use for neutron dosimetry. The objectives of the monograph are to discuss systematic errors in neutron dosimetry with ion chambers for the purposes of radiation protection, including biological and medical applications, and to suggest common procedures. Information about the characteristics of eight different tissue equivalent ionization chambers is included in the present monograph. A number of ion chambers have been checked at the neutron irradiation facilities of GSF Neuherberg and TNO Rijswijk and the results of the tests are reported. A separate review is concerned with the experimental techniques to measure small currents in ion chambers. The ionization in the gas-filled cavity has to be related to the absorbed dose in the surrounding medium. The cavity theory and its practical implications for neutron dosimetry with ion chambers are discussed.

Reviews about kerma values, stopping power, W values and k_u, the relative neutron sensitivity of photon dosimeters, are included. In order to establish the actual dose distribution in the various organs, the practical kerma values in different biological materials are summarized. The finite size of ion chambers can present a serious problem when they are used to measure the dose distribution over the human phantom. The effective points of measurement for in-phantom measurements with photons and neutrons are discussed. The conclusion contains a discussion on the adoption of a common set of basic physical parameters and the characteristics of the neutron dosimetry systems to be used for on-site dosimetry intercomparisons.

EUR Report 6782 EN, 1980, 351 pages, to be ordered through:

Harwood Academic Publishers
37/38 Chancery Lane, London WC2A 1EL, Great Britain
ISBN: 3-7186-0048-X

Price: HFL 115,00

RADIATION PROTECTION - No 18

DEVELOPMENT AND TESTING OF THE TANDEM DOSE EQUIVALENT RATE METER FOR BETA AND PHOTON RADIATION TO BE USED IN RADIATION PROTECTION

J. BOEHM, K. HOHLFELD

Physikalisch-technische
Bundesanstalt
Braunschweig

The Commission of the European Communities is concerned to ensure the protection of workers and the general public from the dangers of ionizing radiation by laying down basic safety standards and by promoting developments to improve practical radiation protection.

The aim of this study was to develop a device which satisfied the practical radiation protection requirements laid down in the Euratom basic safety standards.

The study should be of interest to all those who are responsible for practical radiation protection.

EUR 6834 DE/EN - 1980

Price: BFR 120 - FF 17,40 - HFL 8,20 - USD 4,30 - DKR 23,30 - IRL 2 - UKL 1,80 - DM 7,50 - LIT 3600

Office for Official Publications of the European Communities
B.P. 1003 - Luxembourg

A critical review of nuclear accident dosimeters

B. MAJBORN

Report prepared by
Risø National Laboratory
Roskilde - Denmark

Pursuant to the Treaty establishing The European Atomic Energy Community, the Commission has the task of laying down basic safety standards for the protection of the health of workers and the general public against the dangers arising from ionizing radiation.

The basic safety standards call also for the determination of the dose and committed dose in the event of accidental exposures.

Although experience has shown that accidental exposures are very unlikely, it is necessary to verify the reliability of the methods used.

This study surveys the state of the art of accident dosimetry up to mid-1979; it will also be of interest to groups other than those responsible for radiation protection.

EUR 6838 EN - 1980

Price: BFR 140 - DM 8,80 - LIT 4200 - USD 4,95 - DKR 27,20 -
FF 20,30 - HFL 9,60 -IRL 2,35 - UKL 2,10

Office for Official Publications of the European Communities
B.P. 1003 - Luxembourg

Behaviour of transuranics in the aquatic environment and sediment-water exchanges ; techniques for identifying speciation

Proceedings of the CEC/IAEA joint technical meeting held in Ispra, 24-28 March 1980.

Twenty-six articles were presented in the three main topics areas :

- methods for the determination of transuranic chemical speciation at environmental levels
- methodologies for studying bioavailability of transuranics in aquatic organisms
- chemical methods for the determination of metal fractions and applicability to fresh, estuarine and coastal sediments.

The working papers were followed by technical group discussions on chemical speciation, bioavailability and sediment-water interactions.
Working reports and recommandations prepared by the three technical groups will be published in an IAEA technical report series.

Available : 1981.
To be ordered through the Division of Publications

I.A.E.A.
P.O. Box 100
A-1400 VIENNA

7th Symposium on Microdosimetry, Oxford, 8-12 September 1980

The Commission of the European Communities in collaboration with the National Radiological Protection Board, Harwell, United Kingdom, organized the 7th Symposium on Microdosimetry in Oxford from 8 to 12 September 1980.

The proceedings contain all scientific contributions and the round table discussion and give quite a complete picture of the actual situation of microdosimetry research and its application in the fields of radio-biology and -chemistry, of radiotherapy and -diagnosis, and above all in radiological protection. In 120 papers relevant research results have been presented on the following subject groups:

- physical principles of radiation interaction and energy deposition, spatial and temporal distributions of radiation interaction processes, experimental and mathematical determination of energy deposition spectra,
- problems of radiation quality and its distribution within phantoms and the human body, relations between microdosimetric descriptions of radiation quality of mixed radiations and the mean quality factor,
- dependence of biomolecular and radio-chemical effects and kinetics on radiation quality and their explanation in terms of primary energy deposition processes,
- dependence of biological effects on external radiation and incorporated radionuclides and their interpretation in terms of microdosimetry, influence of local distribution and nature of sensitive targets,
- analysis and interpretation of dose-effect relationships including epidemiological data, in terms of radiation effect mechanisms and their relevance to radiological protection,
- discussion of models of radiation mechanisms with particular regard to the influence of radiation quality and biological repair processes on dose effect relationships.

Proceedings available beginning 1981. For information:

C.E.C.
Biology, Radiation Protection and
Medical Research
D.G. XII

200, rue de la Loi
B-1049 Brussels

European Seminar on Radiation Protection Quantities for External Exposure
Braunschweig, Federal Republic of Germany, 13-15 October 1980.

The proceedings contain 18 papers and a round table discussion of the seminar which was organized by the Commission of the European Communities (CEC) in collaboration with the Physikalisch-Technische Bundesanstalt (PTB), Braunschweig, with the Institut für Strahlenschutz of the Gesellschaft für Strahlen- und Umweltforschung mbH München (GSF), Neuherberg, and with the Institut für Medizinische Physik und Biophysik of the Universität Göttingen.

During recent years there have been important changes and new proposals of radiation protection concepts, which have had a strong impact on radiation quantities. In addition, the change to SI-units resulted in the foreseen demise of the radiation quantity exposure, and it is still not clear which quantity is going to replace it.

In recognizing the need for an internationally consistent solution, and being faced with inconsistencies among the various proposals for appropriate operational quantities, this seminar had been planned.

The programme embraced the following subject groups:

1) Quantities used for defining basic and derived radiation protection limits (their purposes and their interrelationships).

2) Concepts of operational quantities (purposes, theoretical considerations, calculated and measured data).

3) Relationships between operational quantities and quantities used for specifying primary protection limits (i.e. effective dose equivalent, dose equivalent for specified tissues, mean whole body dose equivalent).

4) Measurement and calibration problems associated with operational quantities.

5) Purpose and function of operational quantities as seen from the demands of practical radiation protection.

Proceedings available beginning 1981. For information:

C.E.C.
Biology, Radiation Protection and
Medical Research
D.G. XII

200, rue de la Loi
B-1049 Brussels

Bone and Bone-seeking Radionuclides : Physiology, Dosimetry and Effects.

Proceedings of the Symposium organized by the European Late Effects Project Group (EULEP) and the Commission at Rotterdam, 29 August 1980.

9 papers have been collected on the following subjects :

1. An outline on bone tumor induction and its significance; a review on bone cells and on construction and reconstruction of bone.

2. Under the heading of modern methods for studying the detailed uptake of radionuclides in bone 3 papers have been presented : Cytological studies of plutonium; the spatial distribution of plutonium-239 in bone, the initial uptake and its subsequent changes and dosimetry of alpha-emitting in bone.

3. Concerning the quantitative and biological aspects of bone tumor induction by ionizing radiations 3 further papers deal with bone dose and tumor induction; biological factors as illustrated in work with Strontium-90 and biological factors as illustrated in work with short-lived alpha-emitters.

Edited by V. Volf

Published by Harwood Academic Publishers Ltd.
Chansitor House, 37/38 Chancery Lane
GB-London WC 2A 7EL, 1981, 150 pages
To be ordered through your bookseller or at the Publisher's address.

B

RADIOLOGICAL PROTECTION DATA

– Results of environmental radioactivity measurements in the Member States of the European Community for

air - deposition - water	1973 - 1974
milk	1972 - 1973 - 1974

The present document is the fifteenth report published by the Health and Safety Directorate of the Commission of the European Communities concerning ambient radioactivity and using the data collected by the stations in charge of the surveillance of the environmental radioactivity in Member States. The results are compiled and extracted from the data sent to the Commission in application of Article 36 of the Treaty of Rome instituting the European Atomic Energy Community.

It is the first document in which data from Denmark, Ireland and the United Kingdom which joined the European Community on 1 January 1973 are included in addition to data from Belgium, the Federal Republic of Germany, France, Italy, Luxembourg and the Netherlands.

The results presented in this report cover the years 1973 and 1974 for air, deposition and surface water and the years 1972, 1973 and 1974 for milk.

Eur Report 5630 dk/d/e/f/i/n, 1977, 255 pages, to be ordered through

Office for Official Publications of the European Communities
Boîte Postale 1003 - Luxembourg

FB 600,- DK 94,45 DM 38,70 FF 80,- Lit. 14.300 Fl 40,40 £ 9,60 $ 16,-

Review of existing information on external radiation from natural radioactivity in Europe.

This report reviews the information available for the Member States of the European Communities, together with Austria and Switzerland, as regards external exposure, both indoors and outdoors, from cosmic and terrestrial sources of natural radiation. It discusses the implications of various methods of measurement and highlights present deficiencies in the data. Proposals for obtaining more comprehensive, reliable information are outlined but, in practical terms, provisional isodose maps have already been established and useful comparisons with the environmental radiation exposure resulting from the nuclear industry are already possible.

Internal Doc. nr V-4024/77, e, d, f, 1977, 109 pages,
Available at : Commission of the European Communities,
Directorate General "Employment and Social Affairs"
Health and Safety Directorate
Bâtiment Jean Monnet, A-2/078
Luxembourg - Kirchberg.

Radiological protection - no 15
Results of environmental radioactivity measurements in the Member States of the European Community for air - deposition - water - milk.

The present document is the seventeenth report published by the Health and Safety Directorate of the Commission of the European Communities concerning ambient radioactivity. It was drawn up using the data collected by the stations responsible for environmental radioactivity monitoring in the Member States. The results are extracts from the data sent to the Commission in application of Article 36 of the Treaty of Rome establishing the European Atomic Energy Community.

The results presented in this report deal with radioactive contamination of the air, precipitation and fallout, surface water and milk during 1977 in the nine Member States of the European Community, viz. Belgium, Denmark, the Federal Republic of Germany, France, Italy, Ireland, Luxembourg, the Netherlands and the United Kingdom.

The results are presented in four main sections:

- artificial radioactivity in the air at ground level,
- artificial radioactivity in precipitation and fallout,
- radioactive contamination of water,
- radioactive contamination of milk.

The report also contains supplementary data on short-lived radioelements detected during the fourth quarter of 1977, the list of sampling stations and laboratories together with a list of publications by Member States in this field.

This report places special emphasis on the measurement results for specific radionuclides, but it also contains data on total beta activity so as to ensure continuity vis-à-vis previous reports and provide comparative values.

EUR 6212 DA/DE/EN/FR/IT/NL 1978

290 pages.

Office for Official Publications
of the European Communities

Boîte Postale 1003 - Luxembourg

Price: 750 BFR - 132 DKR - 47,50 DM - 109 FF - 21 200 LIT - 51,50 HFL - 12.60 UKL - 25 USD

Radiological protection - no 17
Results of Environmental Radioactivity Measurements
in the Member States of the European Community for
Air - Deposition - Water - Milk

This document is the 18th report on ambient radioactivity published by the Health and Safety Directorate of the Commission of the European Communities. It was drawn up using the data collected by the stations responsible for environmental radioactivity monitoring in the Member States. The results are extracts from the data sent to the Commission under Article 36 of the Treaty of Rome establishing the European Atomic Energy Community.

The results presented in this report deal with radioactive contamination of air, deposition, surface water and milk during 1978 in the nine Member States of the European Community, viz. Belgium, Denmark, the Federal Republic of Germany, France Italy, Ireland, Luxembourg, the Netherlands and the United Kingdom.

The results are presented under four main headings:
- artificial radioactivity in the air at ground level;
- artificial radioactivity in deposition;
- radioactive contamination of water;
- radioactive contamination of milk.

The report also contains supplementary data on short-lived radioelements detected in the fourth quarter of 1978, the list of sampling stations and laboratories, together with a list of publications by Member States in this filed.

This report places special emphasis on the measurements results for specific radionuclides, but it also contains data on total beta activity so as to ensure continuity vis-à-vis previous reports and provide comparative values.

EUR 6620 DA/DE/EN/FR/IT/NL 1980

Office for Official Publications in the European Communities
B.P. 1003 - Luxembourg

Price: BFR 840 - DM 51,90 - LIT 2400 - USD 30 - DKR 161,80 -
FF 121,40 - HFL 57,20 - IRL 14 - UKL 13

C.

OTHER PUBLICATIONS

Catalogue of Contracts on the Radiation Protection Programme

Containing information on the scientific content of the projects which make up the contract programmes, and on their administrative features such as contractor, duration, budget, etc.. The aim pursued through its publication is to convey a better transparency to the Commission's programme, and to serve as an aid for its management.

For the convenience of the reader, contracts in the main part of the catalogue are systematically assigned to six major sections i.e. dosimetry, radioactive contamination of the environment, genetic effects, short-term and long-term somatic effects of ionizing radiation and evaluation of the radiation risk. In some cases where a contract is composed of several projects belonging to different sections, it is categorized according to its main research area. Therefore, a "list of research subjects" is given in the appendix where the relation between contracts and detailed subjects of the programme is clearly indicated. Furthermore, tables are added with details e.g. about geographical distribution of localities where research is performed, and on the organizations and institutions to which the contractors belong. Finally a list of the scientific research group leaders is given. The catalogue refers to the situation in October 1977. *

Available at : C.E.C.
Biology, Radiation Protection and Medical Research
D.G. XII
200, rue de la Loi
B-1049 BRUSSELS.

* Revised edition in 1979.

Catalogue of Contracts on the Commission's Radiation Protection Programme (September 1979)

Containing information on the scientific content of the projects which make up the contract programme, and on their administrative features such as contractor, duration, budget, etc. The aim pursued through its publication is to convey a better transparency to the Commission's programme, and to serve as an aid for its management.

This catalogue is the second edition and contains the changes that have been made to the 1976-1980 contract programme since the first edition (December 1977). It describes the status as of September 1979.

Available at :

C.E.C.
Biology, Radiation Protection
and Medical Research
D.G. XII
200, rue de la Loi
B-1049 BRUSSELS

Radiation Protection Programme, 1980-1984.
Research Priorities and Scientific Documentation (October 1979).

The Commission of the European Community has prepared a new multiannual research programme in the field of Biology - Health Protection concerning radiation protection and covering the period 1980-1984. The Council of Ministers agreed on that programme in December 1979.

The first part of this booklet contains the official proposal for the framework of the scientific programme as it was transmitted from the Commission to the Council of Ministers. It underlines the priorities derived from the present and foreseeable needs in radiation protection. In order to provide an overall view the research priorities have been grouped into six major sectors : radiation dosimetry and its interpretation, behaviour and control of radionuclides in the environment, short-term somatic, late somatic and genetic effects of ionizing radiation and evaluation of radiation risks.

The second part presents a documentation which is the outcome of the various ways in which the Commission has worked with the scientific community and assembled its views and opinions. As it is published it should give a detailed insight into the aims of the new Community Radiation Protection Programme and help interested institutions and scientists to prepare research projects and to improve joint planning and coordination.

Available at :

C.E.C.
Biology, Radiation Protection
and Medical Research
D.G. XII
200, rue de la Loi
B-1049 BRUSSELS

VII.

VERZEICHNIS DER FORSCHUNGSGRUPPENLEITER

LIST OF RESEARCH GROUP LEADERS

INDEX DES CHEFS DE GROUPES DE RECHERCHE

VII. RESEARCH GROUP LEADERS

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* Bericht noch nicht verfügbar/Report not yet available/
Rapport pas encore disponible.

Navn _____

Institut _____

_____

Gade, nr. _____

Postnummer, sted, land _____

Fordelingskoden er tilpasset stralingsbeskyttelsesprogrammets forskellige arbejdsområder. De rubrikker, der svarer til Deres interessefelter, bedes forsynet med et X.

- ☐ 1. Radioaktiv miljøforurening.
- ☐ 2. Genetiske virkninger af stråling.
- ☐ 3. Strålingsvirkninger på kort sigt, akut strålingssyndrom og dets behandling.
- ☐ 4. Strålingsvirkninger på lang sigt og inkorporerede radionukleiders toksikologi.
- ☐ 5. Strålingsmåling og dens fortolkning; dosimetri.
- ☐ 6. Vurdering af strålingsrisici.

Såfremt De er interesseret i at blive optaget pa vor forsendelsesliste, bedes De tilbagesende dette kort i udfyldt stand (maskinskrevet).

Falls Sie daran interessiert sind, in unsere Versandliste aufgenommen zu werden, schicken Sie uns bitte diese Karte vollständig ausgefüllt zurück (Maschinenschrift).

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Institut _____

_____

Straße, Nr. _____

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Der Verteiler-Code ist den verschiedenen Tätigkeitsbereichen des Programms Strahlenschutz angepaßt. Bitte die Felder ankreuzen, die Ihren Interessengebieten entsprechen:

- ☐ 1. Radioaktive Kontamination der Umwelt.
- ☐ 2. Genetische Strahlenwirkungen.
- ☐ 3. Frühwirkungen bei Bestrahlung, akutes Strahlensyndrom und seine Behandlung.
- ☐ 4. Spätwirkungen bei Bestrahlung und Toxikologie inkorporierter Radionuklide.
- ☐ 5. Strahlenmessung und ihre Interpretation, Dosimetrie.
- ☐ 6. Abschätzung des Strahlenrisikos.

KOMMISSIONEN FOR DE EUROPÆISKE FÆLLESSKABER

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