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EURATOM

Årsberetning 1975

PROGRAM BIOLOGI - SUNDHEDSBESKYTTELSE

Jahresbericht 1975

PROGRAMM BIOLOGIE - GESUNDHEITSSCHUTZ

Annual Report 1975

PROGRAMME BIOLOGY - HEALTH PROTECTION

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PROGRAMME BIOLOGIE - PROTECTION SANITAIRE

Relazione Annuale 1975

PROGRAMMA BIOLOGIA - PROTEZIONE SANITARIA

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- ☐ 5. Misura delle radiazioni e loro interpretazione, dosimetria.
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The annual reports in this volume were prepared under the responsibility of the heads of the research teams, set up under the various contracts, and were submitted in this form to the Commission and its contractual partners.

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Report 1975 arrived late, see end of volume II.
Rapport 1975 arrivé en retard, voir fin du volume II.

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Dk

INDLEDNING

Denne årlige beretning for 1975 indeholder et resumé af de videnskabelige resultater, som er opnået i løbet af det sidste år af femårsprogrammet "Biologi - Sundhedsbeskyttelse", vedtaget i 1971. Denne periode har været kendetegnet af flere begivenheder, hvis virkning vil gøre sig bemærket ved de fremtidige forskningsarbejder. Det vil først og fremmest være hensigtsmæssigt at nævne udvidelsen af fællesskabsforskningen ved de nye medlemsstaters tiltrædelse. Dernæst har beslutningen om at tilkende kerneenergien en vigtigere rolle i medlemsstaternes energiforsyning gjort problemerne i forbindelse med strålingsbeskyttelse særdeles aktuelle, idet disse problemer fremkalder en levende interesse i offentligheden. Endelig skal det anføres, at der ved fastlæggelsen af prioriteter inden for forskningssektoren ligeledes må tages hensyn til visse økonomiske begrænsninger.

De videnskabelige resultater fra året 1975 udgør en anselig sum af nye kundskaber. Inden for sektoren "Strålingsbeskyttelse" råder de beslutningstagende myndigheder, takket være disse resultater, over mere komplette og mere pålidelige data med henblik på at løse de forskellige konkrete problemer, der melder sig, og som netop giver anledning til, at offentligheden sætter spørgsmålstegn ved kerneenergiens fremtid. Opnåelsen af sådanne resultater har kun været mulig ved samarbejde mellem næsten 400 videnskabsmænd, herunder dem fra Kommissionen, inden for mere end 100 forskningsgrupper, laboratorier og institutioner i medlemsstaterne, et samarbejde, som har vist sig frugtbringende for alle de deltagende laboratorier og for hver af medlemsstaterne.

Inden for sektoren "Anvendelse af kerneteknik i den landbrugsvidenskabelige forskning" skal vi blot nævne et enkelt eksempel, som vidner om de fremskridt, der er gjort med anvendelsen af radiogenetiske metoder ved bekæmpelsen af skadelige insekter: de positive resultater af pilotforsøget med selektiv bekæmpelse af ceratitis capitata pa oen Procida.

Forslaget til et nyt femårsprogram "Biologi - Sundhedsbeskyttelse", forelagt Ministerrådet af Kommissionen i 1975, tager i endnu højere grad hensyn til udviklingen i Fællesskabets socio-økonomiske behov. For så vidt angår programmets afsnit "Strålingsbeskyttelse" omfatter forslaget arbejder på videnskabeligt og teknisk grundlag med henblik på udarbejdelse af normer for strålingsbeskyttelse af arbejdstagerne og befolkningen; forskning vedrørende de biologiske og økologiske virkninger af radionuklider og ioniserende strålinger, såvel som forskningsemner, der tager sigte på at forebygge eller begrænse eventuelle skader forårsaget af ioniserende strålinger og at udelukke følgesygdomme heraf. Programmets andet afsnit "Anvendelse af kerneteknik i den landbrugsvidenskabelige forskning" har til formål at forbedre effektiviteten af levnedsmiddelproduktionen samtidig med så vidt muligt at begrænse anvendelsen af pesticider, antibiotica og andre stoffer, som ville kunne have skadelige virkninger på menneskets sundhed.

Inden for rammerne af det nye forskningsprogram bør kontinuiteten i de videnskabelige arbejder sikres i den udstrækning, de frembyder en fællesskabsinteresse. Det forskningspotentiel, som er resultatet af et europæisk samarbejde, der især har udviklet sig mere intensivt i løbet af den seneste femårsperiode, vil blive udnyttet mest muligt til virkeliggørelse af de fremtidige mål.

F. VAN HOECK. P. RECHT.

D

EINLEITUNG

Der vorliegende Jahresbericht 1975 enthält in Kurzfassung die wissenschaftlichen Ergebnisse, die im letzten Jahr des seit 1971 laufenden Fünfjahresprogramms "Biologie - Gesundheitsschutz" gewonnen wurden. Dieser Zeitraum war durch mehrere Ereignisse gekennzeichnet, deren Auswirkung auch auf die künftige Forschungsarbeit zu spüren sein wird. Als erstes ist die Erweiterung der Gemeinschaftsforschung durch den Beitritt der neuen Mitgliedstaaten zu nennen. Sodann erhielten durch die Entscheidung, der Kernenergie eine bedeutendere Rolle in der Energieversorgung der Mitgliedstaaten zuzuerkennen, die Probleme des Strahlenschutzes ein hohes Mass an Aktualität und öffentlichem Interesse. Schliesslich muss die Festlegung der Prioritäten in der Forschung auch bestimmten wirtschaftlichen Sachzwängen Rechnung tragen.

Die wissenschaftlichen Ergebnisse des Jahres 1975 stellen eine beachtliche Summe neuer Erkenntnisse dar. Auf dem Sektor Strahlenschutz werden damit den zur Entscheidung berufenen Instanzen umfassendere und zuverlässigere Daten an die Hand gegeben zur Lösung der anstehenden konkreten Probleme, die auch in der Öffentlichkeit die Frage nach der Zukunft der Kernenergie aufwerfen. Möglich wurden diese Ergebnisse erst durch die Zusammenarbeit von nahezu 400 Wissenschaftlern - darunter auch wissenschaftliche Mitarbeiter der Kommission - in über 100 Forschungsgruppen, Instituten und Einrichtungen der Mitgliedstaaten; eine Zusammenarbeit, die sich für alle teilnehmenden Institute und für jeden der Mitgliedstaaten als fruchtbar erwiesen hat.

Auf dem Sektor "Anwendungen kerntechnischer Verfahren in der landwirtschaftlichen Forschung" soll nur ein Beispiel genannt werden, das bezeugt, welche Fortschritte mit dem Einsatz der Strahlentechnik zur Schädlingsbekämpfung erzielt werden konnten, nämlich die positiven Ergebnisse des Testversuchs der selektiven Bekämpfung der Mittelmeer-Fruchtfliege (Ceratitis Capitata) auf der Insel Procida.

Der von der Kommission während des Jahres 1975 dem Ministerrat vorgelegte Vorschlag für ein neues Fünfjahresprogramm "Biologie - Gesundheitsschutz", berücksichtigt in stärkerem Masse die zunehmenden sozialen und wirtschaftlichen Bedürfnisse der Gemeinschaft. Der Vorschlag umfasst für den Programmabschnitt "Strahlenschutz" Arbeiten über die wissenschaftlichen und technischen Grundlagen zur Festlegung der Normen für den Strahlenschutz der Arbeitskräfte und der Bevölkerung, Untersuchungen über die biologischen und ökologischen Wirkungen von Radionukliden und ionisierenden Strahlungen sowie eine Reihe von Forschungsvorhaben über die Verhütung oder Begrenzung von gegebenenfalls durch ionisierende Strahlungen verursachten Schäden und die Beseitigung ihrer Folgen. Der zweite Programmabschnitt "Anwendungen kerntechnischer Verfahren in der landwirtschaftlichen Forschung" hat zum Ziel, die Effizienz der Nahrungsmittelproduktion zu steigern, aber gleichzeitig die Verwendung von Pestiziden, Antibiotika und ähnlichen Stoffen mit eventuellen gesundheitsschädlichen Wirkungen soweit wie möglich einzuschränken.

Im Rahmen des neuen Forschungsprogramms soll die Kontinuität der wissenschaftlichen Arbeiten, soweit sie von Gemeinschaftsinteresse sind, gesichert werden und das besonders während des letzten Fünfjahreszeitraums durch verstärkte europäische Kooperation gebildete Forschungspotential für die zukünftigen Forschungsaufgaben genutzt werden.

F. VAN HOECK P. RECHT

INTRODUCTION

This annual report for 1975 contains a summary of the scientific results achieved during the final year of the five-year "Biology - Health Protection" programme adopted in 1971. The period was marked by a number of events which will have an impact on future research. First and foremost, there was the enlargement of Community research brought about by the accession of the new Member States. Secondly, the decision to give a more important role to nuclear energy in safeguarding the energy supplies of the Member States has highlighted the problems of radiation protection, which is a subject of keen interest to the public. Finally, there are certain economic constraints that have to be taken into account in determining research priorities.

The scientific results for 1975 add up to an appreciable body of new knowledge. In the sector of "Radiation Protection", for instance, the decision-making authorities now have at their disposal fuller and more reliable data with which to deal with the various practical problems that arise - problems which, in the mind of the public, raise a question-mark over the future of nuclear energy. These results could only be achieved with the collaboration of nearly 400 scientists - including Commission staff - working in more than 100 research groups, laboratories and institutes in the Member States; it was a cooperative effort that proved fruitful for all the participating laboratories and for each of the Member States.

Regarding the "Application of Nuclear Techniques to Agricultural Research", we will quote just one example from this area of research which illustrates the progress made in the control of noxious insects by the use of radiogenetic techniques, namely the positive results of the pilot test in the selective campaign against the Mediterranean fruit fly (ceratitis capitata) on the island of Procida.

In the proposal for a new five-year "Biology - Health Protection" programme, which was presented to the Council of Ministers in 1975, the Commission has still given closer attention to the changing socio-economic needs of the Community. In the "Radiation Protection" sector of the programme, provision is made for the definition of standards of radiation protection for workers and the population on the basis of scientific and technical data; research on the biological and ecological effects of radionuclides and ionizing radiation; and research aimed at the prevention or limitation of damage caused by ionizing radiation, and the elimination of its after-effects. The second sector of the programme "Application of Nuclear Techniques to Agricultural Research" is concerned with ways and means of achieving more efficient food production, while at the same time ensuring the minimum use of pesticides, antibiotics and other substances that could have harmful effects on human health.

In the context of the new research programme, there should be an assurance of continuity in any scientific research of interest to the Community. The research potential that has been built up by closer European cooperation over the past five years will be utilized to the full for the achievement of future objectives.

F. VAN HOECK P. RECHT

F

INTRODUCTION

Le présent rapport annuel 1975 contient un résumé des résultats scientifiques obtenus au cours de la dernière année du programme quinquennal "Biologie - Protection sanitaire" adopté en 1971. Cette période a été caractérisée par plusieurs évènements dont l'effet se fera sentir sur les futurs travaux de recherche. En premier lieu, il convient de citer l'élargissement de la recherche communautaire par l'adhésion des nouveaux Etats membres. En deuxième lieu, la décision de reconnaître à l'énergie nucléaire un rôle plus important dans l'approvisionnement énergétique des Etats membres a rendu très actuels les problèmes de la radioprotection, qui suscitent un vif intérêt dans l'opinion publique. Finalement l'établissement des priorités dans le secteur de la recherche doit aussi tenir compte de certaines contraintes économiques.

Les résultats scientifiques de l'année 1975 représentent une somme notable de connaissances nouvelles. Dans le secteur de la "Radioprotection", grâce à ces résultats, les autorités appelées à prendre des décisions disposent de données plus complètes et plus fiables, en vue de résoudre les divers problèmes concrets qui se posent et sur lesquels l'opinion publique se pose précisément des questions sur l'avenir de l'énergie nucléaire. De tels résultats n'ont été rendus possible que par la collaboration de près de 400 scientifiques y compris ceux de la Commission, au sein de plus de 100 groupes de recherche, laboratoires et institutions des Etats membres, coopération qui s'est révélée fructueuse pour l'ensemble des laboratoires participants et pour chacun des Etats membres.

Dans le secteur "Applications des techniques nucléaires à la recherche agronomique" nous ne citerons qu'un exemple, qui témoigne des progrès réalisés par l'emploi de méthodes radiogénétiques pour la lutte contre les insectes nuisibles : les résultats positifs de l'essai-pilote de la lutte sélective contre la mouche des fruits (Ceratitis capitata) dans l'île de Procida.

La proposition d'un nouveau programme quinquennal "Biologie - Protection sanitaire", présentée en 1975 au Conseil de Ministres par la Commission tient compte davantage de l'évolution des besoins socio-économiques de la Communauté. La proposition comprend, en ce qui concerne le secteur "Radioprotection" du programme, des travaux sur les bases scientifiques et techniques d'élaboration des normes de radioprotection des travailleurs et de la population; des recherches sur les effets biologiques et écologiques des radionuoléides et des rayonnements ionisants, ainsi que des sujets de recherche visant à prévenir ou à limiter les dommages éventuellement causés par les rayonnements ionisants et à en éliminer les séquelles. Le deuxième secteur du programme, "Applications des techniques nucléaires à la recherche agronomique" a pour objet d'améliorer l'efficacité de la production de denrées alimentaires, tout en limitant le plus possible, l'utilisation de pesticides, d'antibiotiques et d'autres substances qui pourraient avoir des effets nocifs pour la santé de l'homme.

Dans le cadre du nouveau programme de recherche, la continuité des travaux scientifiques, dans la mesure où ils offriront un intérêt communautaire, devrait être assurée. Le potentiel de recherche, issu d'une coopération européenne qui s'est surtout développée d'une manière plus intensive au cours de la dernière période quinquennale, sera utilisé au maximum pour la réalisation des futurs objectifs.

F. VAN HOECK. P. RECHT.

INTRODUZIONE

Il presente rapporto annuale contiene un riassunto dei risultati scientifici ottenuti nel corso del 1975, ultimo anno del programma quinquennale "Biologia-Protezione sanitaria" adottato nel 1971. Il periodo è stato caratterizzato da diversi avvenimenti i cui effetti si faranno sentire sui futuri lavori di ricerca. In primo luogo, dobbiamo tener presente l'estensione della ricerca comunitaria in seguito all'adesione dei nuovi Stati membri. In secondo luogo, la decisione di riconoscere all'energia nucleare un ruolo più importante nell'approvvigionamento energetico degli Stati membri ha reso molto attuali i problemi della protezione nucleare, che riscuotono una viva attenzione presso l'opinione pubblica. Infine, nello stabilire le priorità per il settore della ricerca si deve tener conto anche di certe necessità economiche.

I risultati scientifici del 1975 rappresentano una somma considerevole di nuove conoscenze. Nel settore della "Protezione dalle radiazioni" le autorità competenti disporranno, grazie a questi risultati, di dati più completi e più attendibili per risolvere diversi problemi concreti sui quali l'opinione pubblica s'interroga in relazione all'avvenire dell'energia nucleare. E' stato possibile giungere a tali risultati a seguito della collaborazione di circa 400 scienziati, compresi quelli della Commissione, che hanno operato nell'ambito di più di 100 gruppi di ricerca, laboratori ed istituzioni degli Stati membri. Questa collaborazione si è rivelata vantaggiosa per l'insieme dei laboratori partecipanti e per ciascuno degli Stati membri.

Nel settore "Applicazioni delle tecniche nucleari alla ricerca agronomica" citeremo come esempio che testimonia dei progressi realizzati con l'impiego dei metodi radiogenetici per la lotta contro gli insetti nocivi l'esperimento pilota della lotta selettiva contro la ceratitis capitata nell'isola di Procida.

La proposta di un nuovo programma quinquennale "Biologia-Protezione sanitaria", presentata nel 1975 al Consiglio dei Ministri dalla Commissione tiene maggiormente conto dell'evoluzione delle esigenze socio-economiche della Comunità. La proposta comprende, per quanto concerne il settore "Protezione nucleare" del programma, lavori su basi scientifiche e tecniche d'elaborazione delle norme di radioprotezione dei lavoratori e della popolazione; ricerche sugli effetti biologici ed ecologici dei radionuclidi e delle radiazioni ionizzanti, oltre a ricerche per la prevenzione o la limitazione dei danni causati eventualmente dalle radiazioni ionizzanti e per l'eliminazione delle loro conseguenze. Il secondo settore del programma, "Applicazione delle tecniche nucleari alla ricerca agronomica" ha per oggetto il miglioramento dell'efficacia della produzione delle derrate alimentari, limitando il più possibile l'utilizzazione degli antiparassitari, degli antibiotici e di altre sostanze che potrebbero avere effetti nocivi per la salute dell'uomo.

Nel quadro del nuovo programma di ricerca dovrebbe essere assicurata la continuità dei lavori scientifici in ragione del loro interesse comunitario. Il potenziale di ricerca scaturito da una cooperazione europea sviluppatasi più intensamente soprattutto nel corso dell'ultimo quinquennio sarà utilizzato al massimo per la realizzazione dei futuri obiettivi.

F. VAN HOECK P.HECHT

INLEIDING

Het onderhavige jaarverslag over 1975 bevat een overzicht van de wetenschappelijke resultaten die zijn verkregen tijdens het laatste jaar van het in 1971 goedgekeurde vijfjarenprogramma inzake "Biologie - Bescherming van de gezondheid". In deze periode hebben talrijke gebeurtenissen plaatsgevonden die een weerslag zullen hebben op de toekomstige onderzoekwerkzaamheden. In de eerste plaats dient melding te worden gemaakt van de uitbreiding van het communautaire onderzoek door de toetreding van nieuwe lid-staten. In de tweede plaats heeft de beslissing om de kernenergie een grotere rol te laten spelen in de energievoorziening van de lid-staten de vraagstukken inzake stralingsbescherming, die bij de bevolking een levendige belangstelling opwekken, in een zeer scherp daglicht gesteld. Tenslotte dient bij de vaststelling van prioriteiten op het gebied van onderzoek eveneens rekening te worden gehouden met een aantal beperkingen in economisch opzicht.

De wetenschappelijke resultaten die in 1975 zijn verkregen vormen een belangrijke som van nieuwe kennis. Met betrekking tot de "Stralingsbescherming" beschikken de instanties die verantwoordelijk zijn voor de besluitvorming dank zij deze resultaten over vollediger en betrouwbaarder gegevens om concrete vraagstukken op te lossen die zich op dit gebied voordoen en ten aanzien waarvan bij de bevolking vragen rijzen over de toekomst van de kernenergie. Dat men dergelijke resultaten heeft bereikt, is ongetwijfeld te danken aan de medewerking van bijna 400 onderzoekers, waaronder die van de Commissie, in het kader van meer dan 100 onderzoekteams, laboratoria en instituten van de lid-staten. Deze medewerking is zowel voor de deelnemende laboratoria als voor elke lid-staat afzonderlijk bijzonder vruchtbaar gebleken.

Wat de "Toepassing van nucleaire technieken in het landbouwkundig onderzoek" betreft, vermelden wij slechts één voorbeeld dat de vooruitgang illustreert die is geboekt bij het gebruik van radiogenetische methoden ter bestrijding van schadelijke insecten : de positieve resultaten van het proefproject met betrekking tot de selectieve bestrijding van de Middellandsezeevlieg (ceratitis capitata) op het eiland Procida.

In het voorstel voor een nieuw vijfjarenprogramma inzake "Biologie - Besoherming van de gezondheid", dat door de Commissie in 1975 bij de Raad van Ministers is ingediend, is in ruimere mate rekening gehouden met de ontwikkeling van de sociale en economische behoeften in de Gemeenschap. Wat het onderdeel "Stralingsbescherming" van dit programma betreft, hebben de voorgestelde werkzaamheden betrekking op het leggen van de wetenschappelijke en technische grondslagen voor de vaststelling van basisnormen inzake de bescherming van de werknemers en de bevolking tegen straling, onderzoekingen betreffende de biologische en ecologische gevolgen van radionucliden en ioniserende straling, alsmede onderzoekprojecten die erop gericht zijn eventueel door ioniserende straling veroorzaakte schade te voorkomen of te beperken en de gevolgen daarvan tegen te gaan. Het tweede onderdeel van dit programma is gewijd aan de "Toepassing van nucleaire technieken in het landbouwkundig onderzoek" en heeft ten doel de doeltreffendheid van de produktie van levensmiddelen te verhogen, terwijl het gebruik van bestrijdingsmiddelen, antibiotica en andere stoffen die schadelijk kunnen zijn voor de gezondheid van de mens, tot een minimum moet worden beperkt.

In het kader van het nieuwe onderzoekprogramma moet de continuïteit van de wetenschappelijke werkzaamheden, in zoverre zij voor de Gemeenschap van belang zijn, worden gewaarborgd. Het onderzoekpotentieel, dat het resultaat is van een Europese samenwerking die zich vooral in de afgelopen vijf jaar krachtig heeft ontwikkeld, moet optimaal worden benut ten einde de toekomstige doelstellingen te verwezenlijken.

F. VAN HOECK

P. RECHT

Mitglieder im Jahr 1975 des Beratenden Programmausschusses
"BIOLOGIE - GESUNDHEITSSCHUTZ"

Members in 1975 of the Advisory Committee on Programme Management
"BIOLOGY - HEALTH PROTECTION"

Membres en 1975 du Comité consultatif en matière de gestion de programmes
"BIOLOGIE - PROTECTION SANITAIRE"

BELGIQUE - BELGIE

M. ERRERA
A. LAFONTAINE (Chairman)
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O. VANDERBORGHT

BUNDESREPUBLIK DEUTSCHLAND

H.H. BRIESKORN
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J. MASTERSON
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ITALIA

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M. MITTEMPERGHER
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L. STRACKEE
D.W. VAN BEKKUM
G. WANSINK

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G.W. DOLPHIN
Sir Edward E. POCHIN
N.G. STEWART

COMMISSION

A.J. BERTINCHAMPS
P. RECHT
F. VAN HOECK

Secrétariat (Commission):

H.G. EBERT
H. SCHIBILLA

III.

FORSCHUNGSTÄTIGKEIT STRAHLENSCHUTZ

RESEARCH IN RADIATION PROTECTION

RECHERCHES EN RADIOPROTECTION

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 Jahresberichten beschrieben:

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

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

094-BIAN	ITAL, Wageningen (De Zeeuw)
131-BIOUK	NRPB, Harwell (Dolphin)
113-BIOC	GSF, Neuherberg (Burger)
"	GSF, Franfurt (Pohlit)
"	M.R.C., London (Vonberg/Bewley)
"	TNO, Rijswijk (Broerse)
"	Neutron Intercomparison Project/ICRU

Biology Group Ispra

Contractor: Radiobiological Institute TNO
Rijswijk, the Netherlands

Contract No.: 101-72-1 BIOC

Head of Research Team: G.W. Barendsen

General Subject of Contract: Evaluation of the biological effectiveness of different types of radiation.

Event size distributions measured with a cylindrical tissue-equivalent proportional counter are measured and evaluated with respect to their value in predicting the Relative Biological Effectiveness (RBE) of beams of fast neutrons of different energies at various positions in and around collimator ducts.

As reported earlier the smallest simulated volume for which undistorted event size spectra can be measured, has a diameter of approximately 0.3 μm. Although for an optimal analysis of energy deposition spectra in relation to RBE, data for considerably smaller volumes would be required, spectra for larger simulated volumes have been shown to yield useful information about changes in radiation quality. Such measurements have been carried out with a simulated volume of 1.2 μm diameter for collimated beams of neutrons of different energies namely 15 MeV, 3 MeV, 0.9 MeV and 0.5 MeV.

Project nr. 1.

Radiobiological Institute TNO, Rijswijk, The Netherlands

Contract No. 101-72-1 BIOC

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

Evaluation of the biological effectiveness of different types of radiation.

Energy deposition distributions measured in collimated beams of fast neutrons of 3 and 15 MeV energy have been demonstrated to show significant variations as a function of the position in the collimated beams with a conical profile as reported in the last annual report.

Measurements of energy deposition distributions have now been performed with a collimator, which was used for a clinical study of fast neutrons involving irradiations of lung metastases in patients. In order to analyse differences as a function of the location in the beam in detail the neutron energy was varied because these differences might become larger with decreasing energy. In addition to data for 15 MeV neutrons, event size distributions for 0.9 and 0.5 MeV energy have therefore been measured.

The 15 MeV neutrons were produced with a modified Texas Nuclear Generator, in which deuterons were accelerated to 280 kV and impinge on a tritium target.

The 0.9 and 0.5 MeV neutrons were produced through the $T(p,n)^3He$ reaction. The protons were accelerated in a double-belt Van De Graaff K2N-3750 positive ion accelerator.

The collimator is constructed from multilayer of steel and polyethylene to a total thickness of 40 cm. The field defining insert of the collimator is made of steel with a reactangular opening of 3 x 3 cm^2 at the target side and 6 x 8 cm^2 at the exit.

Event size distributions have been measured with a T.E. proportional counter for a simulated volume of 1.2 µm of unit density tissue diameter. The distributions for the 15 MeV neutrons demonstrate a relative decrease of events in the region between 50 and 100 keV/µm compared to the distributions measured for the conical collimator for which data were reported previously.

Dose distributions for equal doses at different positions in and outside the beam are presented in figures 1 and 2 for 0.5 and 15 MeV neutrons, respectively.

The distributions presented in figure 1 for 0.5 MeV neutrons show a marked increase of large event sizes at the boundary of the beam. This must be attributed to small angle scattering of neutrons impinging on the inside wall of the collimator duct. These scattered neutrons will not only increase

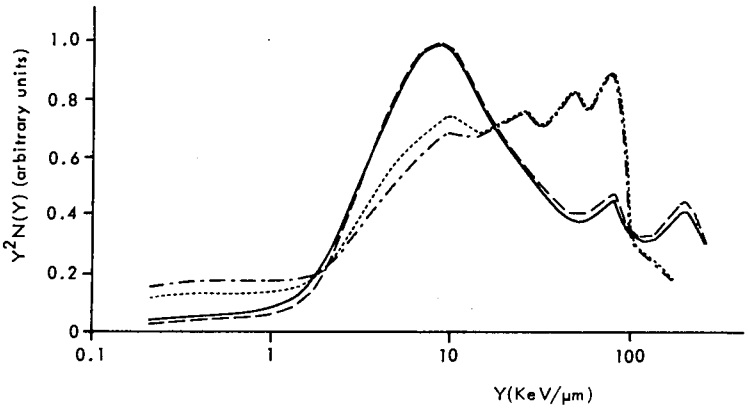

Figure 1. Dose distributions free-in-air of 0.5 MeV collimated neutrons, normalized for equal doses, at different positions.

———— : at centre of the beam

---------- : at boundary of the beam

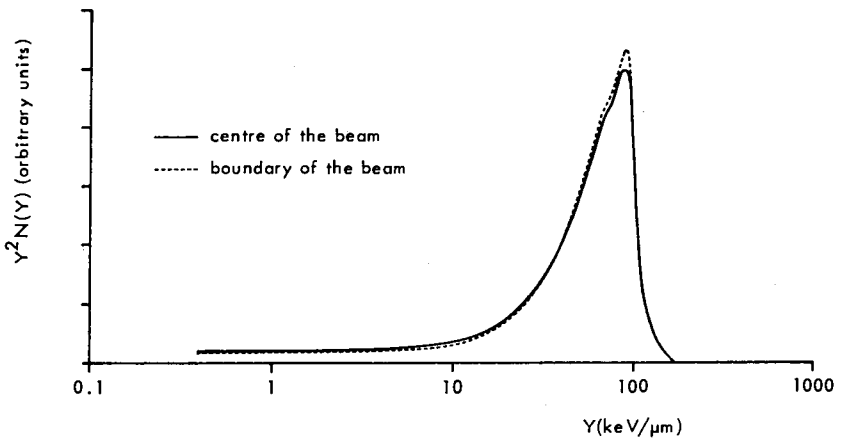

Figure 2. Dose distributions free-in-air of 15 MeV collimated neutrons, normalized for equal doses, at different positions.

———— : at centre of the beam behind the collimator

— — — — : at boundary of the beam behind the collimator

..... : 2 cm of the boundary behind the shielding

-.-.-.- : 4 cm of the boundary behind the shielding

the value of the mean event size, they are also responsible for an increase of the dose rate in the collimated beam compared to the free-in-air beam. In addition they cause for low energy neutrons an increase of the dose rate towards the boundary of the beam as compared to the centre of the beam. The small differences mentioned earlier, measured between spectra for 15 MeV neutrons collimated with the circular and rectangular collimator opening are therefore probably due to similar geometrical differences.

Figure 2 shows that differences in the spectra for the centre and at the boundary of the beam are not significant for these 15 MeV neutrons.

Finally figure 2 shows that outside the beam event size spectra are obtained with a relatively larger contribution of high energy events.

Expected variations in RBE, deduced from these variations in event size distributions will be the subject for further studies.

LIST OF PUBLICATIONS Contract No. 101-72-1 BIOC

Broerse, J.J., and Barendsen, G.W., Radiobiological and physical characteristics of fast neutron beams.
In: Proceedings of the IXth International Cancer Congress, Florence, Italy, 1974. Vol. 5, Surgery, Radiotherapy and Chemotherapy of Cancer, Amsterdam Excerpta Medica, pp. 168-173 (1975).

Hogeweg, B., Variations in energy deposition distributions in collimated neutron beams.
In: Proceedings of the 5th Symposium on Microdosimetry, Verbania Pallanza, Italy, 1975. Commission of the European Communities, in press.

Contractant de la commission : Centre de Physique Atomique
de l'Université Paul Sabatier
118, route de Narbonne, 118
31077 TOULOUSE CEDEX

n° du contrat : 101-72-1-BIOC

Chef du groupe de recherche : D. BLANC

Thème général du contrat : Energy transfer in model substances.

Complete transport simulation of radiotherapy electron and photon beams through accelerator windows, targets, collimators, diffusing screens and biologic tissu has been done and can be applied to many irradiation cases.

An hybrid model for energies between 1 keV and 60 keV is settled up to simulate faithfully electron transport. This method is based on multiple scattering distribution calculations by successive single interaction simulation whatever the type of the interaction. Now first results are available for some gazes such as N_2, O_2, CO_2.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques : J. P. PATAU, M. TERRISSOL, J. P. MANENS.

Titre du projet : Simulation du transport des particules dans la matière par méthode de Monte Carlo. Applications à la dosimétrie.

Dans le domaine d'énergie des accélérateurs linéaires utilisés en radiothérapie (environ 30 MeV maximum) nous poursuivons l'étude de la simulation du transport des électrons et des photons. Nos programmes de calcul mis au point nous permettent de suivre les électrons dès leur émission de l'accélérateur et de simuler leur transport ainsi que celui des électrons secondaires et des photons de freinage émis aussi bien dans la fenêtre de sortie que dans la cible mince interposée, les blindages, les cones compensateurs ou les caches diffuseurs éventuels etc... et enfin le tissu biologique situé derrière. La méthode de Monte Carlo utilisée permet de faire varier tous les paramètres géométriques et physiques des divers éléments et ainsi de comparer ou chercher les meilleures valeurs de ces paramètres en fonction des effets désirés (voir par exemple (1) (2)).

Le problème du transport des électrons d'énergies initiale inférieure ou égale à 1 keV a été résolu au moyen d'une méthode au "coup par coup" précise et fidèle permettant d'obtenir les distributions spatiales de tous les évènements élémentaires et ainsi d'en déduire toutes les quantités intéressantes (pour la méthode et quelques résultats voir (3) et (4)).

Pour des énergies initiales inférieures à 30 keV, les théories de diffusion multiple utilisées perdent de leur précision et l'extension de la méthode au "coup par coup" devenant très longue à exploiter au-dessus de quelques keV, nous avons commencé l'étude d'un modèle hybride à la fois précis et rapide : un modèle de diffusion multiple dans lequel toutes les distributions nécessaires sont obtenues avec la méthode au "coup par coup". Par exemple, sur la figure 1 nous

comparons les distributions de longueurs de trajet pour des électrons d'énergie initiale 30 keV perdant 500 eV dans N_2 obtenues statistiquement par la méthode au "coup par coup" et d'après la théorie de LANDAU modifiée par BLUNCK et LEISEGANG (encore valable dans ce cas précis).

PUBLICATIONS.

1 - JITAO (S.) : "Simulation par la méthode de Monte Carlo de l'influence des cones compensateurs". Doctorat de 3° cycle n° 1708. Université Paul Sabatier, TOULOUSE (1975).

2 - PATAU (J. P.), PANDELLE (R.), TERRISSOL (M.) : "Simulation du transport des électrons de radiothérapie dans un milieu irradié à travers un cache diffuseur". Proceedings of "Biomedical Dosimetry". IAEA-SM-193/12 VIENNE, Mars 1975.

3 - FOURMENTY (J.) : "Simulation sur ordinateur du transport des électrons de basse énergie dans les gaz". Doctorat de 3° cycle n° 1733. Université Paul Sabatier, TOULOUSE (1975).

4 - TERRISSOL (M.), FOURMENTY (J.), PATAU (J. P.) : "Détermination théorique des fonctions microdosimétriques pour des électrons de basse énergie dans les gaz". 5° symposium sur la microdosimetrie, PALLANZA, 24-28 septembre 1975.

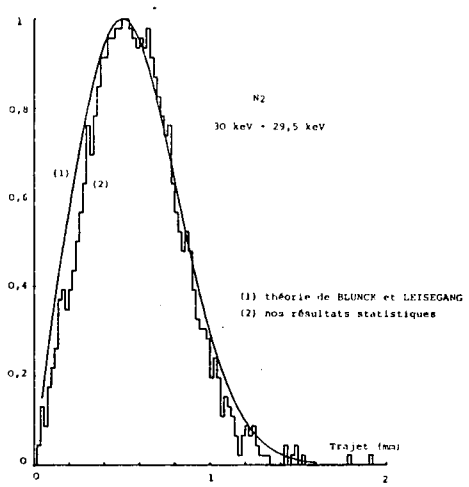

Figure 1 - Distributions des longueurs de trajets pour des électrons de 30 keV perdant 500 eV dans N_2.

Vertragspartner der Kommission: Gesellschaft
für Strahlen- und Umweltforschung m.b.H.,
München

Nummer des Vertrages : 101 - 72 1 BIO C
Leiter der Forschungsgruppe : Prof.Dr.W.Pohlit

Allgemeines Thema des Vertrages:
Dosimetrie in der Mikrobiologie

It is supposed, that in living cells there are radical-induced and direct radiation effects. Their relative contributions to the inactivation of cells depend mainly on the linear energy transfer of the radiation and the environmental structure of the target(s) within irradiated cells.

For the study of the relative contributions of the radical-induced and direct radiation effects on the DNA it is suitable to use aqueous solutions of DNA from different phages. By modifying the radical spectrum it is possible to investigate the relative contributions of the different radicals to the radiation induction of strand breaks in DNA molecules.

In addition to the program for 1975 the contributions of the OH - radicals and the solvated electrons to the whole radiation effect in yeast cells were determined.

Ergebnisse des Projekts Nr. II
Leiter des Projekts und wissenschaftliche Mitarbeiter: Dr.D.Frankenberg, Prof.Dr.W.Pohlit, Dr.M.Frankenberg-Schwager

Titel des Projekts: The role of radicals in the inactivation of biological target molecules

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To determine the contribution of the radicals of the water radiolysis to the induction of strand breaks in DNA molecules radioactively labelled DNA from T2-, T5- und T7-phages were prepared. The purity of the DNA-preparations were checked by sedimentation in a sucrose gradient (5% up to 32 %) using a preparative ultracentrifuge, followed by scanning the sucrose gradient. The results show, that the manipulations in the course of the DNA preparations don't induce strands breaks, which could interfere with the radiation induced strands breaks. In table I are given for different DNA molecules the relative molecular masses, the S-values from literature as well as the experimentally determined S-values. There is an excellent agreement between both S-values. Since the relationship between S-value and relative molecular mass can be described by the formula (FREIFELDER, 1970)

$$S = 2.8 + 0.00834 \cdot M^{0.479} ,$$

it is possible to determine the relative molecular masses of radiation produced DNA fragments by evaluating their S-values.

In addition to the program for 1975 the method developed for aqueous thymine solutions (see EUR 5332, 1974)was extended to determine the relative contributions of OH-radicals and solvated electrons to the whole radiation effect in yeast cells. Suspensions of yeast cells bubbled with N_2O, N_2 and CO_2 with and without the presence of HCOONa were irradiated with 30 MeV electrons. These conditions during irradiation change the radical spectrum within the intracellular water in such a way, that the dose effect curves have to look different from each other,

if water radicals contribute significantly to the radiation effects in yeast cells. However, the results show, that the dose effect curves for the different conditions are equal to each other within experimental error (see fig. 1). The interpretation of these results is, that in yeast cells there are no radiation effects due to radicals of the radiolysis of the intracellular water.

References: D.FREIFELDER, J.Mol.Biol. 54, 567 (1970)

Table I

Relative molecular masses, S-values from literature as well as from experiments for different phage DNA molecules

phage	relative molecular mass	S- value from literature	S- value from experiment
T 2	$108 \cdot 10^6$	61.8 ± 0.4	62.0 ± 1.2
T 5	$67.9 \cdot 10^6$	51.8 ± 0.8	52.0 ± 0.6
T 7	$25.2 \cdot 10^6$	32.0 ± 0.2	31.9 ± 0.2

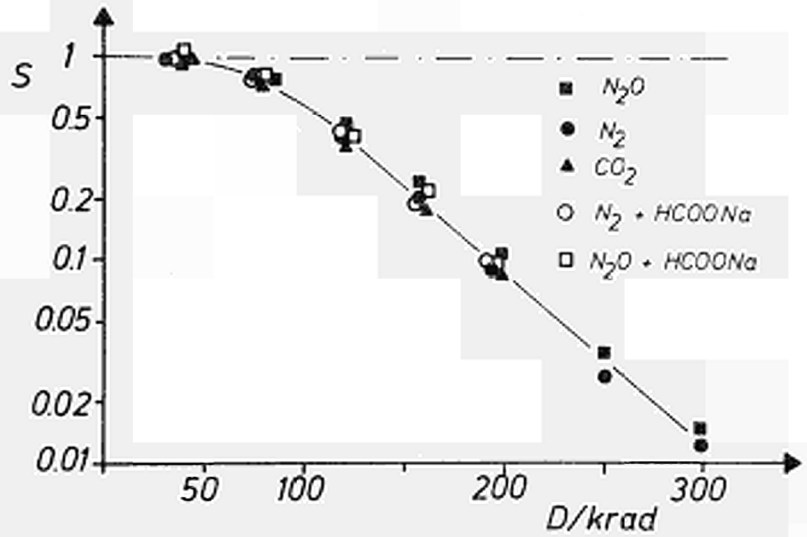

Figure 1 : Dose effect curves for diploid yeast cells irradiated with 30 MeV electrons under different conditions. D: Absorbed dose; S: Survival

Publications:

Frankenberg, D.: Indirect radiation effects related to the environmental structure of targets. Proc. V.Int.Symp.Microdosimetry, EURATOM, 1976

Pohlit, W.: Microdosimetric concepts for indirect radiation reactions. Proc. V.Int.Symp.Microdosimetry, EURATOM, 1976

GESELLSCHAFT FOR STRAHLEN- UND UMWELTFORSCHUNG MBH, MONCHEN
Institut für Strahlenschutz, Neuherberg

Vertrag Nr.: 101 BIOC

Leiter der Forschungsgruppe:
Dr.G.Burger, Prof.Dr.W.Jacobi

Allgemeines Thema des Vertrages: Energy transfer in model substances and radiation effects in condensed matter

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The aim of the investigations is the calculation and measurement of local-energy distributions in irradiated matter. Transport calculations were performed for electrons and energetic ions, to analyze further the correlation between the physical parameters of the radiation and the subsequent physical, chemical and biological effects.

The investigations shall provide the basis for extended research towards a quantitative analysis of dose effect curves for radiation risk assessment at low doses as well as optimal therapeutical use of high LET-radiation.

12 men-month are foreseen for the contract. They were spent mainly for radiation transport studies. The results of experimental microdosimetry are contained in the annual report of contract no. 113 BIOC.

References:

/1/ Varma, M.N., H.G.Paretzke, J.W.Baum. J.T.Lyman and J.Howard
Dose as a function of radial distance from a 930 MeV ion beam
5th symposium on microdosimetry (EURATOM)
Verbania-Pallanza, Italien, 22.-26.9.1975

/2/ Paretzke, H.G.
An appraisal of the relative importance for radiobiology of effects of slow electrons
5th symposium on microdosimetry (EURATOM)
Verbania-Pallanza, Italien, 22.-26.9.1975

Ergebnisse des Projekts

Leiter des Projekts und wissenschaftliche Mitarbeiter:

H.G.Paretzke, G.Burger, G.Leuthold, E.Maier

Titel des Projekts: Radiation Interaction and Energy Dissipation at the Microscopic Level

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The project included two field of investigation

a) Theoretical radiation transport studies

From a 6 month sabbatical stay of one of us (H.G.Paretzke) in the United States there results some interest in very high energetic ions, which may be used for therapy also. Experimental results for the radial ionization profile around a 930 MeV He-ion beam in air were compared with track structure simulation calculations. Only poor data are available on ionizing interactions at rather large beam distances (up to 10 cm in air). It could be estimated that other than nonelastic δ-ray interactions contribute only to 20% at max. of the ionizations measured.

Another subject of interest was the analysis of radiation effects on mammalian cells with respect to the role of the secondary electron distributions. By critical intercomparison of radiobiological data with the corresponding calculations of secondary electrons for the given primary radiations the following results. For primary protons and high energetic light ions the secondary electrons between 0.5 and 2 keV energy seem to play an important role. In case of heavier high LET-ions the primary interactions and low energetic electrons ($\leq$0.5 keV) play the major role.

b) Experimental radiation physics

The two experiments on direct scanning of an ion track in a gas, one measuring the double differential electron flux density, the other measuring the local light output, have been continued. The electronic equipment was completed and calibration measurements started.

Another experiment on the W-values of low energetic electrons was also completed and first measurements were performed in nitrogen gas between 20 and 1000 eV. The results show a monotonic increase of W with decreasing energy. The uncertainty of experimental results is ± 7% at the moment and will be improved.

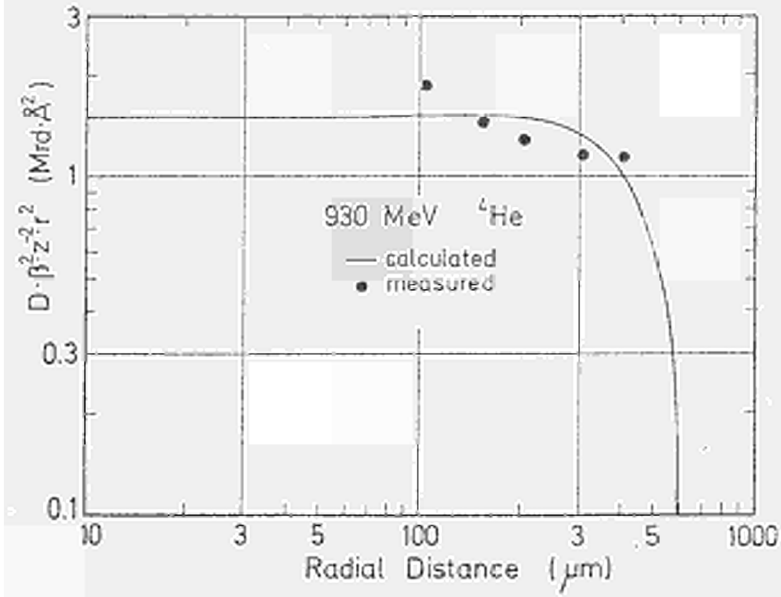

Fig.1 Scaled local dose distribution for 930 MeV He-4 particles in tissue.

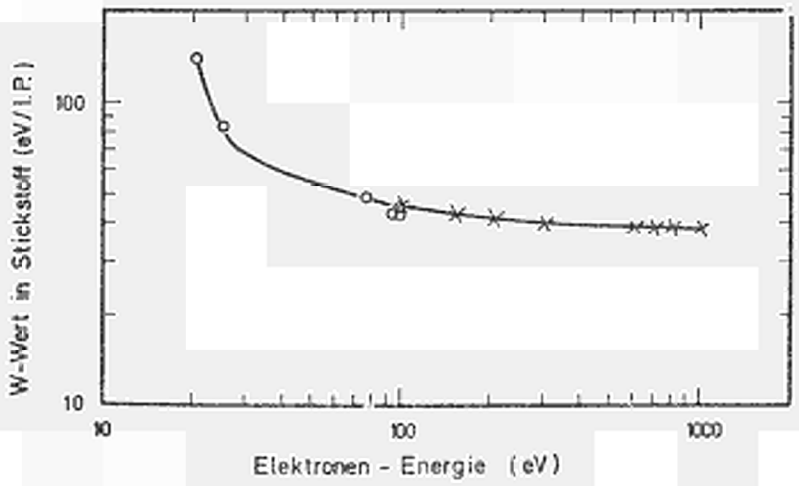

Fig.2 Preliminary results for the W-values of electrons measured in nitrogen gas

- Contractant de la Commission : Université Louis Pasteur - Faculté de Médecine - Laboratoire de Biophysique des Rayonnements et de Méthodologie 11, rue Humann, 67000 Strasbourg
- N° du contrat : 101-72-1-BIOC
- Chef du groupe de recherche : R.V. RECHENMANN
- Thème général du contrat : MICRODOSIMETRY OF CHARGED PARTICLES IN DENSE MATTER.

The introduction of the actual materialized track width in calculations of the δ ray yield as a function of the residual α particle range resulted in a smooth concordance between calculated and measured data. The agreement between experimental and theoretical values in the case of secondary protons distributed along α particle trajectories is very satisfactory provided that the variation of the mean detectable threshold energy $\bar{T}_o$ with the concentration of the CNOH compound is taken into account. The working hypotheses on which were based our studies in this field can therefore be considered as confirmed.

The maximum practical range of medium energy electrons in ionographic emulsions at different concentrations of the CNOH compound has been determined by means of a formula of GRYSINSKI and compared with results obtained by a modified semi-empirical treatment.

PUBLICATIONS.

R.V. RECHENMANN, E. WITTENDORP. Quelques applications de la photographie corpusculaire en microscopie électronique. Jl. Microsc. Biol. Cell, 1975, 23, 20a.

E. WITTENDORP, B. SENGER and R.V. RECHENMANN. Further results in the study of ionizing secondaries emitted along α particle tracks in various hydrogenous media. Proc. 5th Symp. on Microdosimetry, Verbania-Palanza, sept. 1975.

R.V. RECHENMANN and E. WITTENDORP. Basical physical aspects of development of emulsions. Journées d'Etudes sur les Techniques de Radioautographie, Paris, Saclay, oct. 1975; Jl. Microsc. Biol. Cell (in preparation).

RESULTATS du PROJET N°I

- Chef du projet et collaborateurs scientifiques : R.V. RECHENMANN, E. WITTENDORP, B. SENGER.
- Titre du projet : STUDY OF THE ENERGY LOSS PATTERNS OF HEAVY CHARGED PARTICLES.

In order to confirm the validity of our hypotheses that the largest fraction of the secondaries observed along medium and low energy α tracks are short trajectories of secondary electrons and protons, we have compared recent experimental results obtained by means of a refined methodology with calculated data at various concentrations of the CNOH compound, and taking into account the *actual* track width in the different visual detectors used.

δ *rays* : The secondary events with radial spreads from the track axis $r \leqslant 0.35$ μm have been counted along α tracks (E < 11 MeV) recorded in Ilford detectors at different concentrations of the CNOH compound (L4x1, x2, x3, x4, corresponding respectively to 17%, 30%, 36%, 45% in weight of gelatin).

On figure 1a, b, c are represented the histograms resulting from these determinations as well as the calculated data. The latter have been obtained by introducing the variation of the mean threshold energy $\bar{T}_o$, i.e. of the average track width, as a function of the gelatin concentration in our calculations based on a treatment previously described (1).

Indeed, in the case of the standard L4 emulsion, the agreement between calculated and measured yields could be obtained by the introduction in our calculations of a mean threshold energy $\bar{T}_o$ = 6.1 keV.

As far as the diluted emulsions are concerned, a lowering of the average detection threshold energy $\bar{T}_o$ with increasing gelatin concentration had to be expected. Effectively, the best fit for the L4x2 and L4x4 sensitive layers were found to be $\bar{T}_o$ = 5.9 keV and 5.6 keV. This progressive lowering of the detection threshold with the increasing "transparency" of the track as a function of gelatin concentration confirms the validity of our hypothesis that the considered class of secondaries are effectively δ rays. It can also be seen that the number of detectable δ rays diminishes when the AgBr concentration is lowered; indeed, when less heavy atoms are liable to be met by the incoming α particle, the probability of ejection of bound orbitals becomes smaller.

Secondary protons : Considering that a negligible amount of δ rays may be expected below the 3 MeV limit, we counted all the protuberances sticking out of the track core in the track segments corresponding to this energy region.

The histograms representing the experimental yields as a function of

the residual range, for gelatin concentrations corresponding to the dilutions L4x1, x2, x3 and x4, are represented in figure 2a, b, c, d together with the calculated, geometrically corrected, data. The results obtained by means of the LINDHARD formula (2) are in very good agreement with the measured values for the exponent s = 1.46, while the use of the RUTHERFORD formula (3) results in a negligible yield of secondary protons in the energy region considered (Fig. 3).

The energy of a proton emitted at 90° from the trajectory of the incoming α particle and corresponding to a range equivalent to the half width of the track core has been chosen for the lowest detectable proton energy $\bar{T}_o$. In the case of the diluted emulsions L4x2, x3, x4, the mean detectable threshold energy $\bar{T}_o$ has been determined by fitting the calculated yields with the experimental data.

The $\bar{T}_o$ values decrease, like had to be expected, from 10 keV for L4x1 to 9 keV, 8.5 keV and 8 keV for the detectors L4x2, x3 and x4.

The results described above show that a large fraction of the secondaries can be characterized as protons and electrons. Furthermore, it appears that the theoretical treatments applied to the calculation of the yields of the two types of secondaries in the energy region considered of the α particle, lead to an excellent agreement (within the limits imposed by the accuracy of our actual ionographic methodology) between experimental and calculated data. An extension of our determinations to the region confined within the materialized track as well as to other hydrogenous media can therefore be considered as justified. Thus, the calculated yields of secondary protons along medium and low energy α particles can be extrapolated to lower values of $\bar{T}_o$ or to pure gelatin (Fig. 4).

The consistency of all the results obtained by means of the ionographic emulsions at different gelatin concentrations confirms unmistakably the validity of what we considered at the beginning of this work as our two main hypotheses, i.e. that α particles are emitting, even in the lower energy region, a not negligible amount of relatively energetic δ rays and secondary protons.

REFERENCES : 1) AIGUABELLA R., NDOCKO NDONGUE V.B. and RECHENMANN R.V. Proc. 4th Symp. on Microd. (Verbania-Palanza, sept. 1973) N° EUR. 5122 d-e-f (1974), 221. 2) LINDHARD J., NIELSEN V., SCHARFF M. and THOMSEN P.V. Kgl. Danske Vid. Selsk., Mat.-Fys. Medd., Bind. 33, N° 10 (1963), 8. 3) EVANS R.D., The Atomic Nucleus, Mc. Graw-Hill Book Comp. (1967), 849.

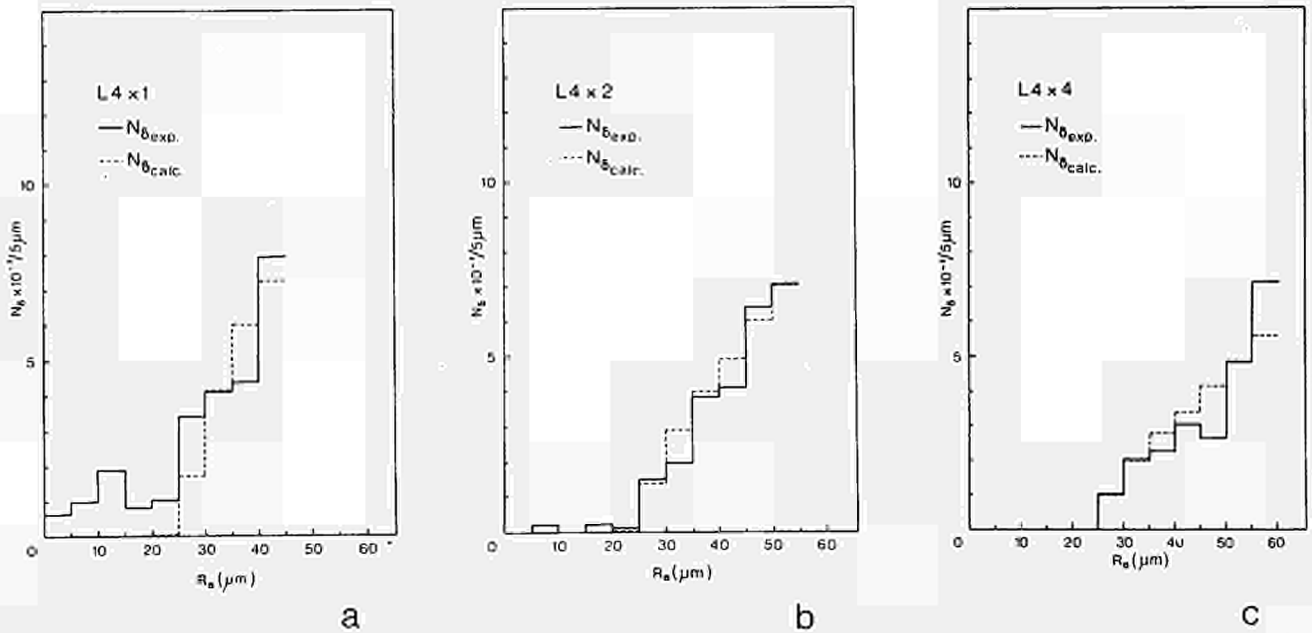

Figure 1. Experimental (——) and calculated (----), geometrically corrected, δ ray yields (per 5 μm) as a function of the α particle's residual range in L4 emulsion. The calculated, geometrically corrected, proton yields corresponding to the considered radial spread (r > 0.15 μm and ≤ 0.35 μm) have been substracted from the experimental data.
L4x1, x2, x4 corresponding to 83%, 70%, 55% AgBr in weight.
The evolution as well in shape as in numerical values with decreasing concentration of the heavy elements Ag and Br is identical for both the calculated and experimental data, provided that the mean detectable threshold energy T_o is correspondingly decreased. The small peak remaining at low energies and at high concentration of the AgBr compound has to be interpreted on the basis of working hypotheses which are not yet considered in this paper.

These data represent only the secondaries with radial projections on the plane of observation exceeding the criterium. The represented values are therefore always smaller than the actual total yields.

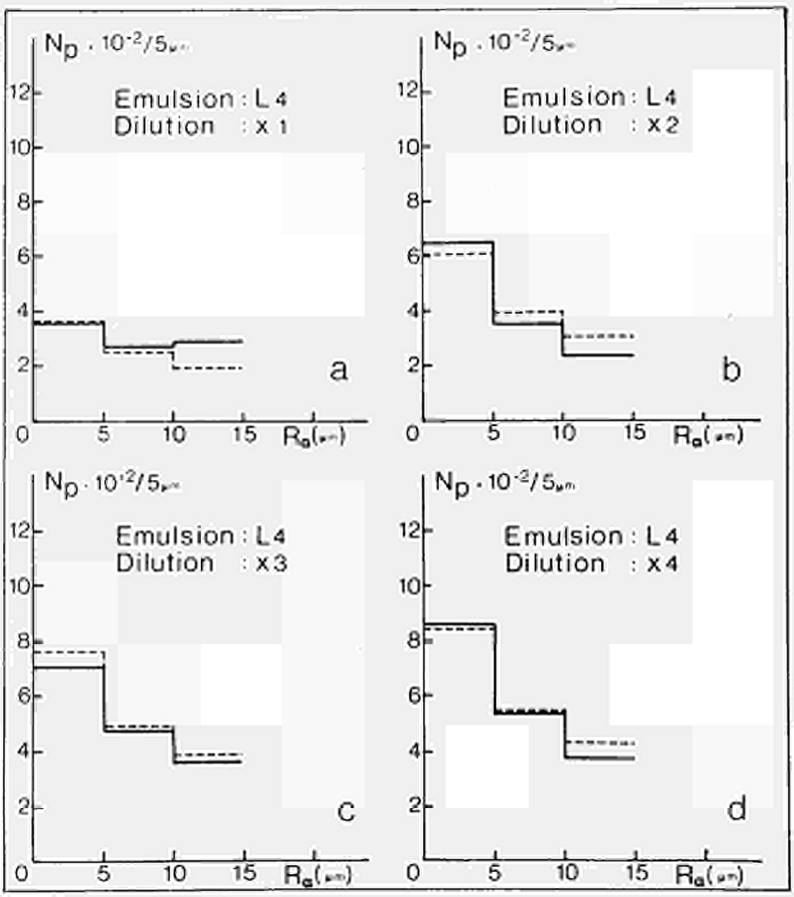

Figure 2. Measured (——) and calculated (----), geometrically corrected, yields (per 5 μm) of secondary protons as a function of the residual range of α particles at different gelatin concentrations of L4 emulsion.

These data represent only the proton tracks with radial projections on the plane of observation exceeding the criterium; the represented values are therefore always smaller than the actual total yields.

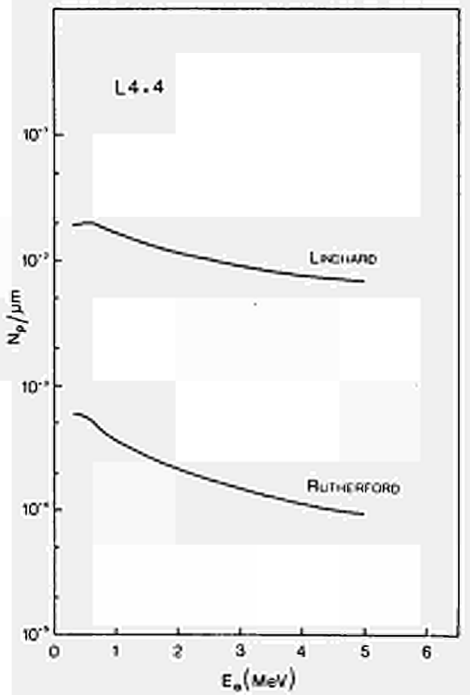

Figure 3. Comparison between the proton yields in L4x4 emulsion, due to the treatment of Rutherford and the number of H-nuclei obtained by means of the Lindhard formula, both corrected in respect to geometry, as a function of the α particle's energy.

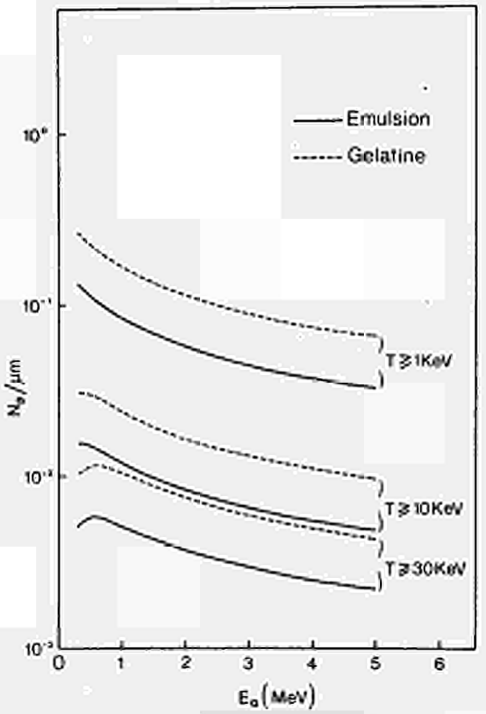

Figure 4. Calculated proton yields corresponding to different threshold energies as a function of the α particle's energy in standard emulsion and in gelatin.

RESULTATS du PROJET N°2

- Chef du projet et collaborateurs scientifiques : R.V. RECHENMANN, B. SENGER and E. WITTENDORP.
- Titre du projet : LOW ENERGY ELECTRONS IN DENSE MATTER.

Till now (1,2,3) our estimations on the range-energy relation of medium energy electrons (1 - 30 keV) have been based on a formula of FELDMAN-BARKAS as well as on a semi-empirical AgBr grain by AgBr grain approach. The results obtained agreed (roughly) with the measured data; they were also applied to the geometrical corrections allowing to determine the actually detectable δ rays along materialized α particle trajectories (4). The good agreement obtained in all these determinations were partly due to the relatively large diameter of the microcrystals.

A more precise determination of the path length of electrons within the energy region considered, crossing ionographic detectors, is actually attempted by means of various approaches. At this time, the application of a treatment of GRYSINSKI (5) to the nuclear emulsion ("standard" formula) has given results which are in good concordance with data obtained by means of a modified semi-empirical, grain by grain, approach mentioned in our annual reports 1973 and 1974 (2,3), like can be seen on figure 1. It appears also that the agreement is satisfactory for the four different concentrations of the CNOH compound.

The formula of FELDMAN-BARKAS (6) which gives satisfactory results for the standard, concentrated, ionographic detectors cannot be applied without modifications in nuclear emulsions with lower AgBr concentrations, while the treatment of GRYSINSKI results in a lengthening of the electron's path when the concentration in the CNOH compound is increased, like had to be expected (Fig. 2).

REFERENCES : 1) RECHENMANN R.V., MELLONI M. and WITTENDORP E. Acta Histochem. Suppl. VIII (1968), 139. 2) RECHENMANN R.V., NDOCKO NDONGUE V.B. and WITTENDORP E. Annual report EUR. 5138 (1973), 31. 3) RECHENMANN R.V., WITTENDORP E. and NDOCKO NDONGUE V.B. Annual Report EUR. 5332 (1974), 26. 4) WITTENDORP E., SENGER B. and RECHENMANN R.V. Proc. 5th Symp. on Microd.. Verbania-Palanza (sept. 1975). 5) GRYSINSKI M. Phys. Rev., 138 (1965), A 336. 6) BARKAS W.H. Nucl. Res. Emuls., Ac. Press (1963), 446. 7) FELDMAN C. Phys. Rev. 117 (1960), 455.

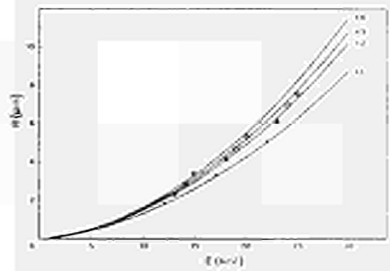

Figure 1. Maximum practical ranges of medium energy electrons as a function of energy in "standard" ionographic detectors at different gelatin concentrations, calculated by means of a formula of GRYSINSKI (5).
x1 : 83 % of AgBr in weight, x2 : 70 %, x3 : 64 %, x4 : 55 %.
The points corresponds to values calculated by means of a modified semi-empirical approach (2,3) :
✱ : x1, ● : x2, ⊡ : x3, ✪ :x4
The agreement is satisfactory for concentrations of the CNOH compound corresponding to the dilutions x1, x2, x3; a slight deviation appears at higher gelatin concentrations.

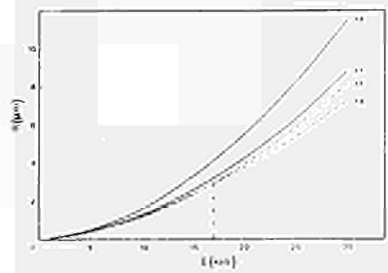

Figure 2. Maximum practical ranges of medium energy electrons as calculated by the formula of GRYSINSKI (5) (——) and by a treatment of FELDMAN (7) (----).
An interesting feature consists in the inversion observed on the dotted curves at 17 keV, while the data obtained by means of the Grysinski's formula correspond to an increase in range with dilution at all the energies considered, like had to be expected.

Vertragspartner der Kommission:

Universität des Saarlandes
Institut für Biophysik

Nr. des Vertrages: 101 - 72 - 1 BIOC

Leiter der Forschungsgruppe: Prof.Dr.H.Muth
Prof.Dr.R.Grillmaier

Allgemeines Thema des Vertrages:

Energy transfer in biological material and model substance

Research work performed in 1975:

I. Exploration of radicals induced by ^{241}Am α-particles in samples of water and DNA-solutions at 77 K. Measurements of dose relationship and mathematical treatment, which at higher doses is of special interest as far as it concerns intramolecular energy transfer. -Conclusions concerning indirect radiation effects - Determinations of the time dependence of radical concentrations at constant temperatures in the period following after irradiation, and treatment by mathematical models, giving some insight into the reaction kinetics which may also be useful for further considerations. -Measurements of radical concentrations at increasing temperatures, enabling comparisons with the temperature dependent behaviour of X-ray induced radicals (influence of LET).

II. The contribution of excitations and ionizations to the primary interaction events of X-rays and the mean energy consumed for producing an ion pair have been determined. It was checked, that the formations of chromosome aberrations in a cell are independent of eachother. The ratios of DNA-radicals and chromosome aberrations (at equal doses) are calculated and the influence of glycin on DNA-radical yield has been investigated (preliminary results).

Ergebnisse des Projekts Nr. .1

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof.Dr.R.Grillmaier, Dipl.-Phys.C.Billotet,Dr.H.Fell

Titel des Projektes: Investigations of the connection of radiation dose, radical production and radiation damage in biological systems (cells) and their components.

I. By investigations of samples of bidistilled water and 1 %-DNA-solutions irradiated at 77 K by alpha-particles and measured at 90 K and higher temperatures by ESR-techniques we have got the following results:

1. a) From 0 to about 60 kRad the numbers of radiation induced radicals increase proportional to radiation dose. At higher doses the increase is reduced (Fig.1). Assuming, that at equal doses the higher amount of unpaired electrons in the DNA solution is due to DNA radicals we have to conclude, that above about 100 kRad the number of DNA radicals nearly remain constant (Fig. 1, lowest curve). This only could be possible, if there is a great fraction of DNA molecules having more than one unpaired electron which compensate their unpaired state in couples by intramolecular energy transfer.

   b) Because the excess of DNA-radicals is much larger as the fraction of DNA-molecules in the solutions, we have to assume, that even at 90 K there exist indirect effects by free electrons and H'-radicals of H_2O-molecules.

   c) The dose relationship of radical concentration (at constant dose rate) is described by an approximative solution of the differential equation $dN(t)/dt=a-bN^2(t)$ where $a_{H_2O}=0{,}875\ 10^{15}\ hrs^{-1}$ $a_{DNA}=1{,}107\ 10^{15}\ hrs^{-1}$ and $b_{H_2O}=0{,}0123^{-15}\ hrs^{-1}$ resp. $b_{DNA}=0{,}0203\ 10^{-15}\ hrs^{-1}$ (N(t)=numbers of radicals at time t after starting irradiation, a=rate of radical production, b=reaction rate of radicals) (Fig.2).

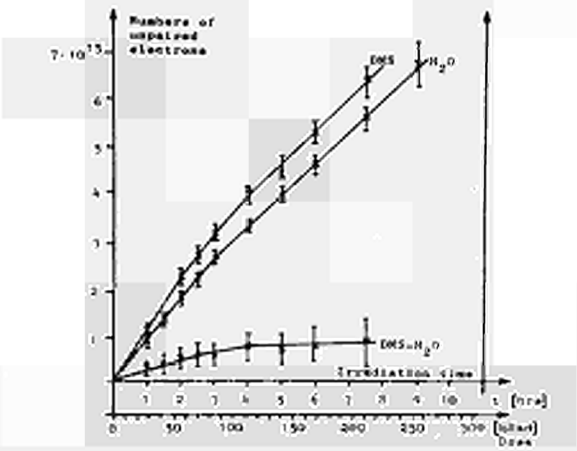

Fig. 1: Alpha ray dose relationship of radicals in H_2O- and DNA samples. DNA-H_2O: difference of radical numbers in DNA- and H_2O samples

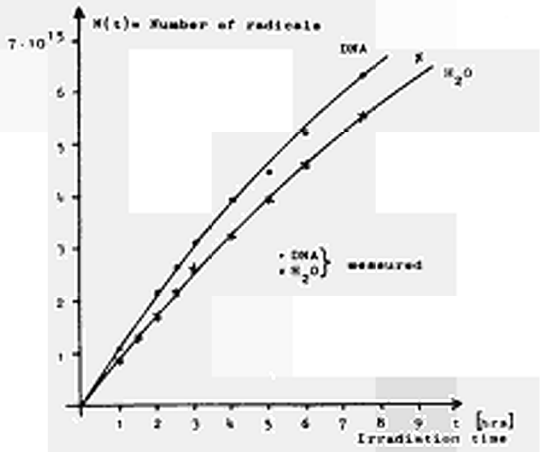

Fig. 2: Comparison of measured and calculated (solid line) dose relationship of radicals induced by α-particles.

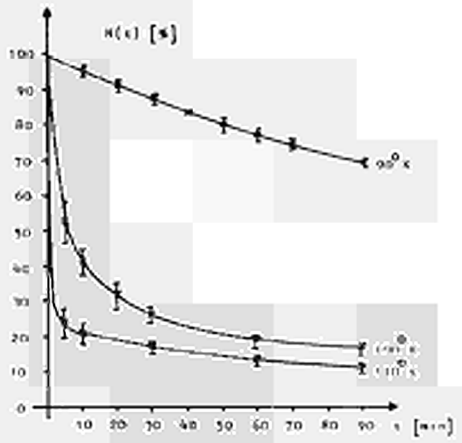

Fig. 3: Time dependence of radical concentration at constant temperatures in the post irradiation period.

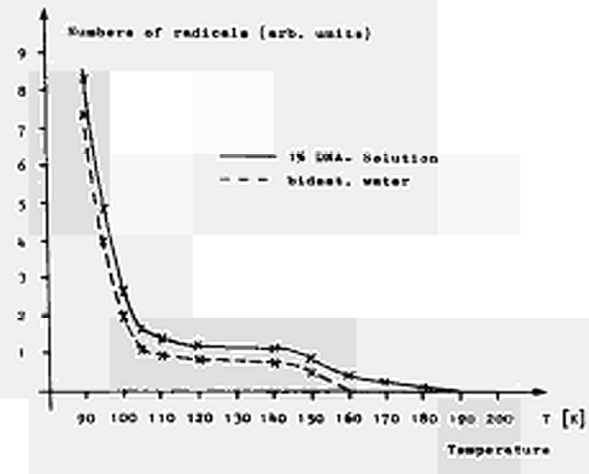

Fig. 4: Numbers (concentration) of radicals at increasing temperatures.

2. To get a view of the types of reactions and reaction rates the time dependence of radical concentration at constant temperatures (T=90, 100 and 110 K) was examined (Fig.3).

   a) The curve measured at 90 K is described by the function $N(t)=(N(0)^{-1}+C(V,v(T),q_s)^{-1}$ which is derived as a solution of the differential equation: $dN(t)/dt=C(V,v(T),q_s)\ N^2(t)$, $C(V,v(T),q_s)=2\cdot\sqrt{2}\ v(T)\ q_s/V$.
   For $C'=C\ \frac{N(0)}{100}$ we found: $C'=4{,}85\ 10^{-5}$
   (t = time after stopping irradiation, v(T) = mean diffusion velocity of radicals at temperature T, q_s = reaction cross section, V = volume occupied by the total amount of radicals)

   b) The curves measured at 100 K and 110 K may only be interpreted if we assume two different types of radicals. The approximative solution $N(t)=N_1(0)\cdot\exp(-\alpha N(t)\cdot t)+N_2(0)\cdot\exp(-\beta N(t)\cdot t)$ of the corresponding system of diff.equations

   $$dN_1(t)/dt=-C_{11}N_1^2(t)-C_{12}N_1(t)\cdot N_2(t)$$
   $$dN_2(t)/dt=-C_{22}N_2^2(t)-C_{12}N_1(t)\cdot N_2(t) \text{ and}$$
   $$N(t) = N_1(t)+N_2(t)$$

   fits the measured curves very well if at T=100K $N(t) = 61{,}5\exp(-4{,}07\ 10^{-3}\ N(t)\cdot t+38{,}5\exp(-6{,}197\ 10^{-4}N(t)\cdot t)$ at T = 110 K:
   $N(t)=76{,}2\exp(-2{,}552\ 10^{-2}N(t)\cdot t)+23{,}8\exp(-6{,}165\ 10^{-4}N(t)\cdot t)$
   The calculations indicate, that about 69 % of the radicals are more mobile as the rest of about 31 %. The relationship between the reaction kinetic coefficients C_{ij} and the factors in above equations has still to be found.

3. There are no quantitative nor qualitative differences between the ESR-spectra of samples irradiated with α-particles and X-rays. But in α-irradiated samples the radical concentrations decrease much faster, when the samples are annealed (Fig.4). The reason surely is the much smaller mean distance between the radicals in the α-irradiated samples.

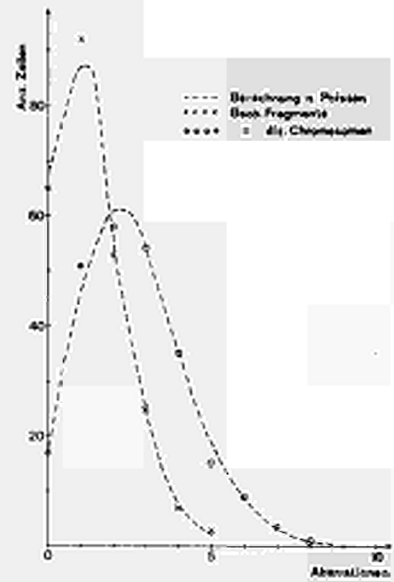

Fig. 5: Frequency distribution of cells (lymphocytes) with different numbers of aberrations.

D (rad)	$\frac{R_z}{B}$
500	$3,9\ 10^5$
1000	1,9
2000	0,9
3000	0,6

Tab. 1: R_z = Number of DNA-radicals (at dose D) in a DNA-quantity equal to the DNA content in a lymphocyte cell.(Measured in frozen DNA solution).
B = Number of chromosome breaks in a lymphocyte cell at dose D.

	H_2O	DNS	DNS + Glycin
100%	87	-	-
1%		98 100 97	117 113 118
0,1%		95 97	105 98 104
0,01%		91 91	98 91 95

T = 87° K

Tab. 2: Numbers of radicals in DNA and DNA + Glycine solutions (arb. units) at 87 K.

	H_2O	DNS	DNS + Glycin
100 %	20	-	-
1 %		37 31 29	44 40
0,1 %		36 34	45 48 44
0,01 %		35 35	40 35 32

T = 130° K

Tab. 3: The same as in table 2 at 130 K.

II.1. a) Investigations of X-irradiated H_2O-samples, irradiated and measured at 4.2 K indicate, that 46 % of the primary radiation events are due to excitation and 54 % to ionizations. The corresponding mean energy producing one ion pair is 51 eV.

b) Using culture medium containing 10 % glycerol we got: 36 % of excitations and 64 % ionizations. The mean energy for one ionpair is 35 eV.

2. Comparing the frequency distributions of cells with 0,1,2 and so on aberrated chromosomes with the Poisson distribution we have verified, that the aberrations are produced independently (Fig. 5) from eachother.

3. Comparing the dose relationship of radical concentrations (measured in pure DNA solutions) with the dose relationship of chromosome aberrations (in human lymphocytes irradiated at the same conditions as the DNA solutions) we have got the figures in table 1. Because we have supposed, that the amount of DNA radicals, induced by indirect effects of e^- and $H^\cdot$, may be reduced if the solution contains additional substances, we have investigated also solutions of glycin and mixtures of DNA and glycin in solutions. The results (table 2 and 3, arb. units) indicate, that the radical concentration in pure glycin solution is not greater as in H_2O samples but in the solution containing both, DNA and glycin, the radical concentration is still greater than in the pure DNA solution (preliminary result).

References:

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GRILLMAIER, R., FELL, H.: Kombinierte Untersuchungen von strahleninduzierten Radikalen und Chromosomenaberrationen.
Fünftes Symposium über Mikrodosimetrie
22. - 26. September 1975, Verbania Pallanza, Italien.

Contractor: United Kingdom Atomic Energy Authority
Atomic Energy Research Establishment, Harwell

Contract No.: 128-74-1 BIOUK

Head of research team: D.H. Peirson

General subject of Contract: NEUTRON AND GAMMA-RAY DOSIMETRY AND MICRODOSIMETRY

This contract is divided into six projects, three of which are concerned with improving neutron dosimetry measurements, (a) with ionisation chambers, (b) in man phantoms and (c) development of new solid state dosimeters. Two more projects are concerned with the measurement of photon spectra in medical diagnosis and for dosimeter calibration. The sixth project is designed to measure track structure using a low pressure cloud chamber.

Project 1. Improved measurements of neutron absorbed dose with ionisation chambers.

Tissue equivalent and other types of homogeneous ionisation chambers are widely used for neutron dosimetry. The aim of the present work is to measure ionisation values in the gases commonly employed in neutron dosimetry for comparison with computed values. To ensure a proper basis for the work, the group participated fully in the European Neutron Dosimetry Intercomparison (ENDIP) under the sponsorship of Euratom.

Project 2. Neutron and LET spectrometry in a man-phantom.

The aim of this work is to improve our knowledge of the penetration of neutrons through the body and hence to provide data on the LET distribution and the dose equivalent at various points in the body. The sensitivity and threshold of the spectrometry system based upon a small stilbene crystal have been established and measurements have been made in a rectangular slab phantom. Theoretical calculations based upon transport and diffusion theory have been made for similar phantoms and the experimental and theoretical results will be compared.

Project 3. Solid state fast neutron dosimeters.

The aim of this project has been to examine two new types of solid state fast neutron dosimeters. Both use the effects of momentum transfer from fast neutrons to ions in solids, and use luminescence techniques to detect the changes in the materials brought about by neutron-ion collisions.

Project 4. Spectra of X-radiation used in medical diagnosis.

The long term 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. Present work is concentrated on determining the effects of various factors on the spectra produced by diagnostic X-ray machines. Also phantoms to simulate accurately parts of the body are important for the assessment of these different spectra when used in radiology. Thus we have determined the attenuation of different tissues in the body as a preliminary to producing such a phantom.

Project 5. Photon spectra for dosimeter calibration.

The aim of this project is to improve the quality of calibration procedures by providing improved spectral information for the radiation used. Since few suitable gamma-ray sources are available for the determination of the energy response of dosimeters, X-ray machines are used to generate pseudo-monoenergetic radiations. In the energy range up to 300 keV filtered X-ray beams are used and up to 100 keV fluorescent radiations are employed. It is important to choose the applied voltage and filtration to ensure that there is minimum extraneous radiation away from the main peak. The spectral measurements are used to check the purity of the spectra.

Project 6. Track structure of ionising radiation using a low pressure cloud chamber.

The aim of the project is to investigate the spatial distribution of ionisation in the tracks of charged particles with the aid of a low pressure cloud chamber. Individual droplets formed on ions produced by low energy electron tracks are easily resolved due to the unique

construction and operating conditions of the chamber. The coordinates of the droplets are measured from stereoscopic pairs of photographs at 90^{o} and hence the position of each droplet is uniquely determined.

Results of Project No. 1

Head of Project and scientific staff: H.J. Delafield
J.A.B. Gibson
P.D. Holt
S.J. Boot

Title of Project: IMPROVED MEASUREMENT OF NEUTRON ABSORBED DOSE WITH IONISATION CHAMBERS

ENDIP measurements

This year the laboratory fully participated in the European Neutron Dosimetry Intercomparison Project (ENDIP) under the sponsorship of Euratom. Mixed field dosimetry measurements were performed at GSF Neuherberg Munchen, this being the location of the intercomparison for those institutes working on neutron radiobiology.

The neutron and gamma-ray components of kerma in tissue were measured in free air under standardised conditions, for monoenergetic neutron beams of nominal energies 0.67, 2.1, 5.5 and 15.5 MeV from a Van de Graaff accelerator, and for fission neutrons emitted by a californium-252 source. The principal measurements were made with a pair of ionisation chambers; one homogeneous with wall and electrodes of conducting plastic of composition $C_n H_n$ and filled with acetylene, and the other with a graphite wall and filled with carbon dioxide. Supporting gamma-ray measurements were made with film and thermoluminescent dosimeters.

Table I

Mixed field dosimetry measurements for ENDIP

Nominal neutron energy MeV	Method of interpretation	*Dose (rad per 10^4 monitor counts or rad h^{-1} for ^{252}Cf		
		Neutron	Gamma-ray	Total (n+γ)
0.67	CH + graphite	11.4	< 0.14	11.4
2.1	CH + graphite	6.38	< 0.11	6.42
5.5	CH + graphite	5.34	0.10	5.43
15.5	CH + film	4.45	< 0.20	4.55
^{252}Cf	CH + graphite	3.90	1.33	5.23

*Provisional estimates of dose as at 31 December 1975. The californium-252 source was measured on 2 July 1975.

Doses derived from the paired CH-plastic and graphite ionisation chambers (CH + graphite) are given in Table I. These results have been derived for the expected neutron spectra based on the bombarding particle energy and the thickness of target for the monoenergetic sources, and for the published californium-252[1] fission spectrum.

The twin chamber technique could be readily applied to measure monoenergetic neutrons of 0.67, 2.1 and 5.5 MeV. The response of the graphite chamber to neutrons in this energy range is small, enabling the gamma-ray contamination to be measured with sufficient accuracy to give the neutron component of dose to a high precision (± 2% standard error, ± 7% systematic error). By contrast the graphite chamber has a significant response to 15.5 MeV neutrons, and moreover an additional uncertainty arises in calculating the response at this energy from the need to estimate the contribution (∿ 20%) to the ionisation from recoils produced in the wall of the chamber. These combined effects result in reducing the accuracy of the measurement of the neutron dose given by the twin chambers. In these circumstances the neutron dose can be derived more accurately (± 7% systematic error) by basing the correction for gamma-radiation on the film dosimeter, (designated (CH + film) in Table I).

This arises because the sensitivity of the film to neutrons though unknown, is much smaller than that of the graphite ionisation chamber, and hence the uncertainty limits on dose using the film are smaller than the error limits using the ionisation chamber.

For the californium-252 source, the total (n+γ) dose was measured with a systematic error of ± 8% by the twin chamber technique. By contrast the determination of the individual components of dose was less accurate being ± 9% for the neutron dose and ± 15% for the gamma-ray dose.

We conclude that the precision of the experimental measurements was high (± 1 to ± 3% standard error), but the technique was limited by systematic errors arising from uncertainties in the W and kerma ratios. A full detailed report will shortly be published.

Reference

1. MEADOWS, J.W. Californium-252 fission neutron spectrum from 0.003 to 15 MeV. Phys. Rev. vol.157, p.1076, 1967.

Results of Project No. 2

Head of Project and scientific staff: P.D. Holt
K.G. Harrison
Mrs A.J. Taylor
J.A.B. Gibson

Title of Project: NEUTRON AND LET SPECTROMETRY IN A MAN-PHANTOM

Development of the organic-scintillator spectrometer for neutrons

A new spectrometer using a high gain photomultiplier tube (EMI type 8850) has been assembled. Pulse shape discrimination is achieved with a new system designed in Electronics and Applied Physics Division at Harwell. The response of a small stilbene crystal (diameter 1 cm, height 1 cm) has been determined and with a light pipe of 30 cm the threshold is 450 keV. With the crystal close to the photomultiplier this threshold is 200 keV. (The gamma ray rejection is set at 3000:1). The response of the detector to proton induced recoils from neutrons has been determined with a series of monoenergetic spectra from the IBIS accelerator at Harwell. The spectrum was determined by a time of flight method and the fluence was measured with a de Pangher long counter. The sensitivity of the crystal is about 0.07 count n^{-1} cm^2 over the neutron energy range 1 to 5 MeV. At 6 MeV the maximum range of proton recoils is about one tenth of the crystal diameter so at energies below this the wall effect should be negligible. Double scattering is also negligible and so the neutron spectrum can be determined from the observed pulse height distribution by simple differentiation.

Measurements in a rectangular phantom

We have concentrated on a study of the neutron spectrum and LET spectrum on the central axis of a rectangular phantom 30 cm x 30 cm x 15 cm filled with water and irradiated by a neutron beam of 1.0 MeV or 6.0 MeV incident perpendicular to the 30 cm x 30 cm face. The neutrons were obtained by the (p,t) reaction on the 6 MeV Van de Graaff and the 14 MeV tandem Van de Graaff; it was considered that a 6 MeV neutron beam produced by the (p,t) reaction would be much less contaminated with extraneous neutrons than one produced using the (d,d) reaction. A spherical helium-3 counter 3.2 cm diameter was used to measure the neutron

spectrum in the phantom for 1 MeV neutrons incident, and an organic scintillator with pulse shape discrimination for 6 MeV neutrons incident. The raw data obtained with these counters are awaiting analysis. The LET (and energy deposition) spectrum at positions inside the phantom was measured in both cases with a half inch diameter tissue equivalent Rossi counter (manufactured by E.G. and G.). These data also are awaiting analysis.

Theoretical calculations of the spectra in rectangular phantoms

The phantom is assumed to be a rectangular block and to be irradiated by a parallel beam of unit flux of monoenergetic neutrons in an arbitrary direction until a steady state is reached. The spectrum after each neutron has undergone two collisions is calculated at each point of a three dimensional lattice; for elastic collisions the proper distributions over angles and energies of the scattered neutrons are taken into account. It is assumed that after these two collisions the flux is isotropic. The diffusion equation is then solved by a finite difference method for each lattice point at a number of energies: the spectrum obtained from the earlier collisions is used as a source. Through the finite differences in the diffusion terms the equations for the lattice points are coupled, but, above thermal energy and if no fissile material is present, collisions do not increase neutron energies. The equations for each energy are therefore coupled only to those for higher energy values and they can be solved successively instead of simultaneously.

Calculations have been done for various amounts of water in rectangular tanks which are irradiated at different angles and with beams of different initial energies.

Typical scattered flux distributions for unit lethargy at some depths of penetration are shown in Figure 1. Here the water is 60 cm x 60 cm x 30 cm and the incident beam is perpendicular. The initial energy is 0.1 MeV. The hydrogen has been treated as a light element, but, because the scattered distribution due to the oxygen varies steeply over small energy intervals it has been replaced in the calculation by a mixture of light and heavy elements in such a way that the average slowing down properties are unchanged. The collisionless remnant of the beam at the

incident energy drops exponentially with penetration from unity at the point of entry. The peaks in the flux distributions for penetrations between 0.7 cm and 5 cm at small energy loss are due to neutrons which have undergone one collision only.

Comparison between theoretical and measured spectra will be made in 1976.

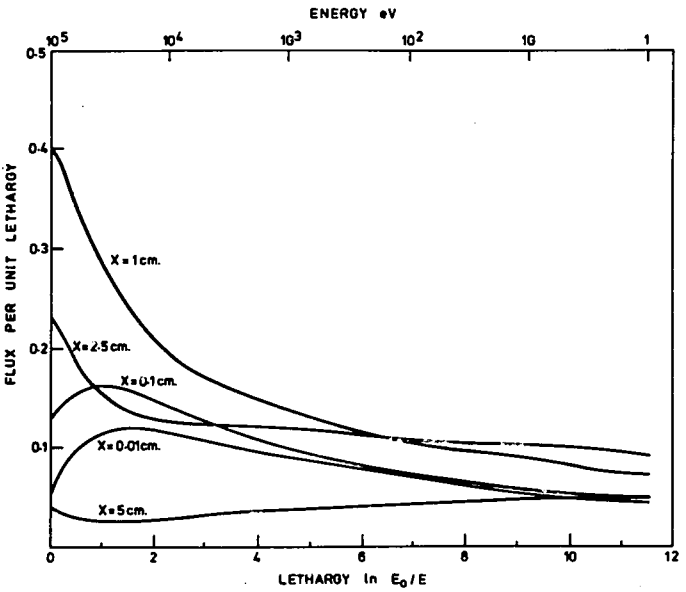

Figure 1

Variation of the flux per unit lethargy with lethargy for various values of the penetration (X). $E_o = 10^5$ e V

Results of Project No. 3

Head of Project and scientific staff: A.E. Hughes
G.P. Pells

Title of Project: SOLID STATE FAST NEUTRON DOSIMETERS

Direct displacement system

Work has continued on using the luminescence of F^+ and F centres in alkaline earth oxides to assess the prospects for a fast neutron dosimeter in which lattice defects induced by neutron-ion collisions are used to monitor dose. In these compounds ionising radiation is known to be ineffective in producing new defects in the crystal structure, but fast neutrons produce oxygen vacancies with one or two trapped electrons (F^+ and F centres respectively), which have their own characteristic optical absorption and emission bands. The number of these defects produced will therefore be proportional to the fast neutron dose, and calculations show that the energy response should be favourable.

The principal objective of work this year has been to establish as clearly as possible the pre-dose luminescence of available crystals of calcium oxide. Using a He-Cd laser to excite F^+ luminescence, it has been found that the background level of luminescence from as-received crystals corresponds to the luminescence produced by fast neutron doses between 30 rads and 300 rads. As far as can be ascertained at present, the background luminescence is characteristic of F^+ centres in the bulk of the material. Even the highest quality single crystals available show a background luminescence which would make it extremely difficult to detect a neutron dose of 10 rads. It has been concluded that this sets a lower limit to the usefulness of this photoluminescent system and, on present evidence, the prospects for improvements are small.

In an effort to bypass the effects of these pre-existing defect centres, the thermoluminescence behaviour of neutron irradiated magnesium oxide and calcium oxide has been investigated. The recombination of vacancies and interstitials produced by neutron irradiation gives rise to a release of stored energy, a small fraction of which should be in the

form of thermoluminescence. It is known that recombination takes place in these materials between 300°C and 500°C, so a thermoluminescence system has been constructed which is capable of working up to 600°C. In magnesium oxide only thermoluminescence from impurity centres activated by ionising radiation has been observed. In calcium oxide there is also intense thermoluminescence from impurity centres, but there is a peak centred at about 400°C which appears to correlate with fast neutron dose. However, it is unfortunately rather weak and overlapped by some of the impurity peaks, so that the prospects for using it in dosimetry do not look good. A lower limit to the detectable dose is being established.

An additional system using the generation of F aggregate centres in lithium fluoride and sodium fluoride has been briefly explored. It has been shown that more F aggregate centres are produced by neutron irradiation than by an equivalent amount of deposited energy delivered by cobalt-60 gamma rays, but even so the rad response to neutrons is a factor of three lower than to gammas. The use of ^{6}LiF could improve this situation, at some sacrifice in energy response characteristics.

Ion injection system

Following the lack of success in fabricating suitable mixtures of fine Gd_2O_3 and SiO_2 powders reported last year, attempts were made to explore some alternative methods of achieving an intimate mixture of source and host compounds with high interfacial area and no inter-diffusion prior to irradiation. In one method powders of Gd_2O_3 were dispersed in a liquid monomer which was then polymerised into a solid plastic pellet. In the second method fine SiO_2 powders were dispersed in molten $Gd(NO_3)_3.5H_2O$ which was then solidified. In both cases some weak Gd^{3+} emission was observed after fabrication, and no evidence could be found for any new detectable injected ions even for fast neutron doses up to 10^6 rads. In view of these consistently negative results the theory of the injection system has been re-examined, including an analysis of the conditions for observing luminescence. It has been concluded that in the relatively opaque manufactured pellets the true interfacial area was about two orders of magnitude lower than ideal for 10 nm crystallites, and that only about one per cent of any luminescence excited in the pellets actually escapes for detection. Under these circumstances it is not

considered that the ion-injection system should be pursued any further and a report on the work is being prepared.

Publication

DATTA, S. and HUGHES, A.E. Luminescence dosimetry using sodium fluoride single crystals. Health Physics vol.29, p.420, 1975 (also available as report AERE - R 7847).

Results of Project No. 4

Head of Project and scientific staff: J.A.B. Gibson
L.H.J. Peaple
M. Marshall
R. Birch
G.M. Ardran
T.J. Crosby

Title of Project: SPECTRA OF X-RADIATION IN MEDICAL DIAGNOSIS

Present work is concentrated on determining the effect of various factors on the spectra produced by diagnostic X-ray machines. These factors include voltage, current and voltage waveform, target angle, target material, filter materials and filter thicknesses. Their effect on radiographic quality can be provisionally estimated using data on the attenuation and scatter characteristics of tissue and the response of the imaging device (type of intensifying screen, film, etc). However final evaluation involves clinical judgement by a radiologist.

A paper outlining the factors affecting the output from diagnostic X-ray generators was presented at the Conference on Biomedical Dosimetry, IAEA, Vienna 1975 (Publication 1).

Spectral variations during the mains waveform cycle

The instantaneous spectra at selected points of the waveform cycle and the variation in photon output during the cycle have been measured, using circuits developed previously, for a nominally constant potential generator and for a half-wave rectified set. The variation of photon flux depends upon the high voltage and filament power supplies, the current and the X-ray filtration. A paper on this work has been submitted for publication.

Spectrum and intensity of off-focus radiation

This radiation, amounting to between 5% and 30% of the exposure, can reduce the quality of the radiographic image, and gives unnecessary dose to the patient.

There is conflicting evidence on its spectral quality compared with that of radiation from the focal spot. For a typical diagnostic tube we

have shown, using X-ray spectrometry, that the extra focal radiation is softer than the radiation from the focal spot.

Effect of target angle

Measurements of the effect of the angle of the X-ray beam to the target have been made. The effect of changes in electron beam angle have not yet been investigated. Present results indicate significant changes in exposure with X-ray angle and small differences in the spectrum shape. The results are being analysed.

Filtration and screens

Possible combinations of X-ray spectra, K-edge filters and intensifying screens which may give improved image quality or reduced dose are being examined. Possible combinations are predicted from measured unfiltered spectra, the attenuation of various filters, tissue absorption and theoretical screen response. Combinations are tested using phantoms and will be finally used with patients.

Attenuation of low energy X-rays in soft tissue and bone

Accurate attenuation coefficients for human tissues at energies below 100 keV are required in the fields of diagnostic radiology and radiation protection. The data is also necessary to ensure that suitable tissue substitutes are selected for the production of realistic body phantoms. Measurements of narrow beam attenuation have been made using highly collimated beams of fluorescent X-rays having energies between 9.9 keV (germanium) and 59.3 keV (tungsten). The use of a high resolution detecting system including a Ge(Li) diode enabled the effects of unwanted X-ray lines and scattered radiation to be excluded.

Improved samples have been prepared for a range of tissues (e.g. muscle, lung and thyroid) using a technique which overcomes the problems of dehydration and deterioration and enables the thickness of the final section to be determined accurately. Measurements have been made of the density of the tissues and the values used to convert the measured coefficients to mass attenuation coefficients.

The absorption measurements on these improved samples covering thirteen different soft tissues together with bone have been completed, the results computed and a preliminary report prepared.

Publications

1. GIBSON, J.A.B., ARDRAN, G.M., PEAPLE, L.H.J., MARSHALL, M., CROOKS, H.E. and BIRCH, R. Standardisation of the output from diagnostic X-ray generators. Biomedical Dosimetry, IAEA, Vienna p.509, 1975.

2. MARSHALL, M., PEAPLE, L.H.J., ARDRAN, G.M. and CROOKS, H.E. A comparison of X-ray spectra and outputs from molybdenum and tungsten targets. Br. J. Radiol. vol.48, p.31, 1975.

Results of Project No. 5

Head of Project and scientific staff: L.H.J. Peaple
T.J. Crosby
J.A.B. Gibson

Title of Project: PHOTON SPECTRA FOR DOSIMETER CALIBRATION

Since few suitable gamma ray sources are available for the determination of the energy response of dosemeters, X-ray generators are used to generate substitute radiations. In the energy region up to 300 keV filtered X-ray beams are employed. The tube voltage and added filter are selected to produce a pseudo monoenergetic beam with the requisite mean energy, spectrum width and associated exposure rate. Below 100 keV fluorescent radiation which is more closely monoenergetic may be employed. The primary X-ray beam is used to excite the characteristic radiation of suitable elements whilst selective filters reduce the k_β with respect to the required k_α radiation. The exposure rate and purity are functions of the X-ray tube voltage and the primary and secondary filtration. Spectrum measurements are necessary to investigate the purity and characteristics of existing and proposed series of reference radiations and to design new ones.

The International Standards Organisation (ISO) fluorescent series contains ten radiations each characterised by the material of its radiator and filter, its thickness and the X-ray tube voltage. In order to produce satisfactory exposure rates large area radiators and filters are required with diameters of say 13 cm and 10 cm respectively. For the elements marked with an asterisk in Table I such foils can be fairly readily obtained.

Table I

Present ISO series

Radiator	Filter	Energy $K_{\alpha 1}$ (keV)
germanium	-	9.89
zirconium*	strontium	15.78
cadmium*	silver*	23.17
caesium	tellurium	30.97
samarium	cerium	40.12
europium	gadolinium	49.13
tungsten*	ytterbium	59.32
gold*	tungsten*	68.81
lead*	gold*	74.97
uranium*	thorium*	98.44

The remainder, it is suggested, may be produced by incorporating suitable compounds, (oxides, carbonates stc), in a plastic binder. The incorporation of a precise quantity of powder in a suitable plastic to produce a uniform large area disc of defined mass per unit area has proved exceptionally difficult. In addition surface stresses caused discs which were originally flat to distort badly. However discs suitable for definitive spectral measurements were produced by the following process. Approximately twice the required quantity of powder was sieved and mixed thoroughly, under vacuum, with a resin plus hardener, poured into a mould and allowed to cure. Both sides of the disc were carefully machined flat and the uniformity and content of the disc determined by absorption measurements using narrow collimated beams of fluorescent radiation. Radiators were machined and measured successively to produce discs with the specified content. The filters were reduced in thickness until the ratio of the β to α lines was close to 0.03 and the content subsequently determined by the same absorption technique. Due allowance was made for the absorption of the test radiation in resin, oxide, carbonate etc. The discs necessary to complete the ISO series have been produced and have proved suitably robust. Machining the discs appears to remove the surface stresses and prevent distortion.

Table II

Proposed series with metallic foils

Radiator	Filter	Energy $k_{\alpha 1}$ (keV)
zinc	-	8.64
molybdenum	zirconium	17.48
tin	silver	25.27
neodymium	cerium	37.36
europium	gadolinium	49.13
tungsten	ytterbium	59.32
gold	tungsten	68.81
lead	gold	74.97
uranium	thorium	98.44

A series shown in Table II which can be obtained in the form of metallic foils has been investigated. A disadvantage is that the large area foils of neodymium, cerium, europium, gadolinium and ytterbium could not be obtained in the UK and had to be obtained from the United States. In addition some of the foils have to be hermetically sealed to prevent oxidation. An advantage is that the absence of any resin etc. reduces scattered radiation and improves the spectral purity.

Exhaustive spectrum measurements have been carried out for the two series using both Ge(Li) and NaI(Tl) detectors the latter system giving a more accurate measure of the effects of scattered radiation above 100 keV. The results are being processed.

Results of Project No. 6

Head of Project and scientific staff: M. Marshall
D.A. Williams

Title of Project: TRACK STRUCTURE OF IONISING RADIATIONS USING A LOW PRESSURE CLOUD CHAMBER

A tissue equivalent gas mixture is used consisting of (approximate pressures in mm of Hg) 19.4 mm hydrogen, 11.3 mm oxygen, 10.1 mm ethanol and 0.8 mm nitrogen. This composition can be readily achieved since only ethanol is significantly absorbed in the lubricating oil of the chamber. By introducing the ethanol first equilibrium at the correct pressure can be obtained before adding the other gases.

A new X-ray tube has had to be designed, made and tested. It is a Coolidge tube with a metal body and is continuously pumped. It should overcome many of the previous problems of sealed glass tubes which tended to go soft and were fragile. This tube is robust and the target, filament and X-ray filters are easily changed. It is now working and photographs of clusters of droplets produced by the interaction of aluminium X-rays will be produced shortly.

A paper on the design, construction and operation of the chamber has been written and will be submitted for publication.

Contractor:	National Radiological Protection Board
Contract No.:	129-74-1 BIO UK
Head of research team(s):	Dr. G. W. Dolphin
General Subject of Contract:	Dosimetry

In project 1 the method for re-estimation of dose in Lithium Fluoride, initially proposed by Dr. E. W. Mason when at NRPB (Mason, 1971), has been developed. This method, employing the u.v. transfer of charge at an elevated temperature, has been examined in detail with a view to producing a routine re-assessment assembly. Exposure of various preparations of Lithium Fluoride to u.v. light at a temperature of 80°C has shown that a significant percentage of the original thermoluminescence response has been recovered. This has allowed re-estimation of doses down to 500 millirad. A detailed examination of the main dosimetry and higher temperature glow curves of various types of thermoluminescent materials is now proceeding. This will enable a deeper understanding of the solid state processes associated with the thermoluminescence response of Lithium Fluoride and other useful phosphors to be developed, as well as establishing their relationship to the deeper trapping centres employed in the re-assessment procedures.

In project 2 further experimental data needed to improve the estimation of bronchial dose from inhaled radon daughters were obtained. Free ions were found to deposit in segmental bronchi with an efficiency of only about 25% of that previously assumed. The reduced diffusion coefficient is ascribed to rapid attachment of water molecules in the upper bronchial airways. Clearance of radon daughter ions from bronchial epithelium to blood has also been measured in vivo in the rabbit.

Results of Project No. 1

Head of Project and scientific staff: B.L. Davies
A. G. Sherwin
C.M.H. Driscoll
D. T. Bartlett

Title of Project: Solid state physics processes underlying the properties of some materials used in thermoluminescence dosimetry

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Re-estimation of Dose in LiF Dosemeters

The technique of dose re-estimation by the method of u.v. transfer at an elevated temperature has been investigated with the object of using the technique routinely. The recalled signal for TLD 700 Lithium Fluoride-Teflon discs is 8 of the original signal and for TLD 700 Lithium Fluoride hot pressed chips it is 14. The response was found to be linear over the dose range 500 millirad to 100 rad. For the re-estimation of an original dose of 1 rad, the standard deviation is 150 millirad.

The Effect of Preparational and Environmental Procedures on the Thermoluminescence Sensitivity of Lithium Fluoride Dosemeters

It has been shown previously (Mason et al., 1974) that, unless correct handling procedures are used with Lithium Fluoride:Teflon dosemeters, discolouration will result in significant reduction in TL sensitivity particularly if organic cleaning liquids are employed. However, repeated annealing of the dosemeters at high temperature was also observed to produce cumulative darkening. Both Lithium Fluoride itself and the presence of impurities in the teflon appear to be factors contributing to this discolouration process. Then possible, therefore, high temperature annealing should be omitted.

The effect of a wide range of controlled temperature, humidity and storage conditions on the TL sensitivity of Lithium Fluoride has shown that the main dosimetry traps in the phosphor are extremely stable under most conditions. Optimum stability of response under storage was found under conditions of ambient temperature but at high relative humidity.

The effects of varying the cooling rate after high temperature anneal on the glow curve peak and shape have been appreciated for some time. However, for the range of cooling rates employed for dosimetry purposes, these effects are unlikely to be of concern.

References

Mason, E.W. Phys. Med. Biol., 16, 303, 1971.

Mason, E.W., Marshall, T.O., Shaw, K.B., Blackman, T.E., Johns, T.F., Preston, H.E. Brit. J. Radiology, 47, 361, 1974.

Publications

Mason, E.W., McKinlay, A.F. and Clark, I. Cooling rate effects in thermoluminescent dosimetry grade lithium fluoride. I. Implications for practical dosimetry. Phys. Med. Biol., 21, January 1976.

Mason, E. W., McKinlay, A. F., Clark, I. and Saunders, D. Elimination of thermoluminescence sensitivity variations in LiF:PTFE dosemeters incurred by improper handling procedures. National Radiological Protection Board Report No.37, 1976.

Results of Project No.2

Head of Project and Scientific Staff: Dr. A. C. James
Miss M. R. Stott
Miss J. Trinder

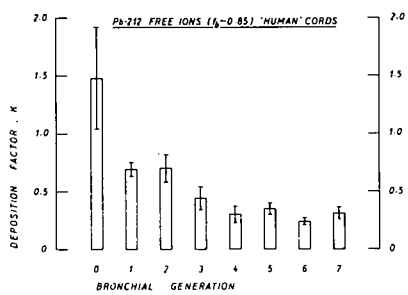
Title of Project: The deposition of radioactive aerosols in lungs

Bronchial deposition of unattached Pb-212 ions

Measurements were described in the 1974 report of local bronchial deposition of Pb-212 ions attached to condensation nuclei, in excised pig lung. It was shown that deposition of nuclei in segmental bronchi is within 25% of that calculated for diffusion from steady, laminar air-flow.

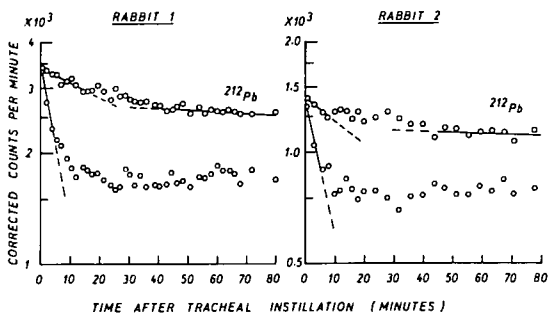
Further measurements of bronchial deposition of unattached Pb-212 ions have been made. Typical results are shown in figure 1, when the ions were inhaled through model human vocal cords. The deposition factor, K, is the ratio of the Pb-212 concentration observed on the bronchial surface to that calculated for an aerosol of diffusion coefficient 0.054 cm^2 sec^{-1}, i.e. the value measured in ambient air. The K factors for segmental bronchi were only about 25%.

It was necessary to establish whether this observation was due to migration of Pb-212 ions from their initial deposition site, as suggested in the 1974 report, or to a reduced efficiency of bronchial deposition. However, instillation of carrier free Pb-212 ions in small volumes of distilled water into segmental bronchi did not show any significant migration of Pb-212 ions into lung parenchyma during prolonged in vitro ventilation followed by dissection. It is therefore concluded that inhaled free ions grow rapidly by attachment of water molecules, into particles with diffusion coefficient of approximately 0.01 cm^2 sec^{-1}, giving rise to a significantly lower efficiency of deposition in segmental bronchi than previously assumed.



In vivo bronchial clearance of radon daughter ions

In order to calculate the α-dose delivered to basal epithelial cells, it is necessary to know whether the radon daughters Po-218, Pb-214 and Bi-214 remain bound to mucus after deposition or diffuse into the bronchial epithelium and bloodstream before radioactive decay. Techniques have been developed for the instillation of Pb-212, Bi-212 and Tl-208 ions in 10 µl distilled water into lobar bronchi in anaesthetised rabbits. Subsequent clearance of this material has been measured by direct external counts of the chest and uptake to blood by regular sampling from the carotid artery. Results of a preliminary series of experiments are shown in figure 2. The data points represent Pb-212 and Tl-208 activity in the lungs of two rabbits followed for periods of 80 minutes. Approximately 10% of the instilled Pb-212 ions diffused to blood with a half-time of about 15 minutes. The majority of Pb-212 ions were cleared with a half-time of about 10 hours. A more rapid clearance of either Bi-212 or Tl-208 would account for the observed change in activity of Tl-208 in the lung. A further series of experiments is being carried out in which higher γ-activities are instilled, to enable Bi-212 clearance to be measured directly by counting the weak 727 keV photopeak.



Publication

James, A.C. Bronchial deposition of free ions and sub-micron particles studied in excised lung. Read at 4th Inter. Symp. on Inhaled Particles and Vapours, Edinburgh, September 1975 (to be published).

Contractor: Central Electricity Generating Board,
Berkeley Nuclear Laboratories,
Berkeley, Gloucestershire, England

Contract No.: 135-74-7 BIOUK

Head of
Research team: Dr B.M.Wheatley

General subject
of contract: Production of intermediate energy neutrons

A description of the novel source of intermediate energy neutrons developed in the 1974 programme has now been published. The source has been used to calibrate eight instrumental systems. Some of the studies have been undertaken in conjunction with workers at the Atomic Energy Research Establishment, Harwell.

Emphasis is now shifting towards a wide-ranging study of all methods of producing high fluxes of intermediate energy neutrons which has identified the most promising system as that utilizing resonance filtration in the beam tube of a high flux reactor. A study of European research reactors has identified those most appropriate for this type of application. A study of cross sectional data and the basic nuclear physics underlying interference cross section minima has allowed a preliminary identification of 19 possible filtration systems giving principle neutron energies ranging from 50eV to 145 keV.

Results of Project number:	135-74-7 BIOUK
Head of project and scientific staff:	J R Harvey A J Mill
Title of project:	Investigation of sources of intermediate energy neutrons

The "½keV" neutron source has been used to calibrate eight instrumental systems which have previously not been calibrated at this energy. They are:-

The Andersson-Braun rem survey instrument.
The Studsvik rem-meter type 2202D.
The de Pangher long counter.
Bonner spheres: 2", 3", 4", 5", 6", 7", 8" diameter.
The Nuclear Enterprises neutron remmeter 0075.
The "Basson" intermediate energy neutron survey meter.
The CEGB albedo dosemeter.
The AEA neptunium fission foil personal dosemeter.

Analysis of the experimental data from the first three calibrations is complete and a paper describing the results has been accepted for publication. The two remmeter instruments have reasonably good rem-dependence at energies around "½keV". The de Pangher long counter has an energy-independent sensitivity to neutron flux from intermediate to fast energies and could therefore be used to monitor intermediate energy neutron flux from other systems such as resonance scatter sources.

Analysis of the experimental data from the other five systems is currently being undertaken.

Of the four basic methods which can be used to produce 'mono-energetic' neutrons at intermediate energies, resonance filtration of a reactor beam has been shown to be the most promising in terms of high neutron flux, low gamma and neutron contamination and a wide range of useful energies. Three natural elements - scandium, iron and silicon already identified by other workers - have suitable windows in their cross-section at 2keV, 24keV and 145keV respectively and together with suitable resonance scatterers may be employed to produce useful mono-energetic beams from a reactor.

Further filters are most likely to be identified amongst even-Z, even-A isotopes, none of which occur naturally as 100% abundance of the element and only a few with abundance greater than 90%. Since there are few cross-section measurements on single isotopes where the cross section is very small, multi-level analysis of the resonances of a number of isotopes have been undertaken in order to identify suitable windows. This has been done using a computer program and several possible windows have been identified (See table).

Table Energies in keV of possible filter windows with approximate width at 300 mb

1. Materials which may be used in elemental form
   2 ± 0.2, 24 ± 1.5, 65 ± 4, 125 ± 4, 145 ± 4.

2. High abundance isotopes
   1.5 ± 0.4, 1.9 ± 0.45, 4 ± 0.3, 12 ± 0.7, 48 ± 2.

3. Low abundance isotopes
   0.05 ± 0.01, 0.07 ± 0.001, 0.15 ± 0.01, 0.23 ± 0.03,
   0.48 ± 0.03, 0.65 ± 0.005, 19 ± 0.1, 45 ± 2, 47 ± 2

A study of research reactors in Europe has identified the seven most appropriate for this application.

A possible system would be based on a filter in a radial tube of one of these reactors. This filter would consist of a single isotope giving beams at a number of energies or a combination of isotopes with a common window giving a single energy. In the first case a secondary scattering foil external to the reactor would probably be necessary to eliminate unwanted neutrons. The flux in the beam external to the reactor would typically be in the range 10^6 - 10^7 $ncm^{-2}s^{-1}$ over an area of say 10 cm^2. The secondary scattering foil would therefore constitute a source of 10^7 - 10^8 n sec^{-1}. The actual yield attainable depends crucially on the amount of filtering material available.

Publication

A neutron source with an effective energy of 0.5 keV.
J. R. Harvey, R. C. Bending
Physics in Medicine and Biology 21.1, 85-97 January 1976

Contractor : National Physical Laboratory,
Teddington, Middlesex, U.K.

Contract No. : 143-74-7 BIOUK

Head of research team : S.C.Ellis

General subject of contract : MEASUREMENT OF THE FERRIC ION YIELD FOR ELECTRON IRRADIATION OF THE FERROUS SULPHATE DOSEMETER

The aim of the project is the measurement of the ferric ion yield, G (Fe^{3+}), for the ferrous sulphate dosemeter (an aerated, aqueous solution of 10^{-3} mol dm^{-3} ferrous sulphate in 0.4 mol dm^{-3} sulphuric acid) for electron energies from 2 to 20 MeV. The present contract for the first stage of the project was for the measurement of G (Fe^{3+}) for 2 MeV electrons.

In the range of electron energies from 2 to 30 MeV the published G (Fe^{3+}) values vary from 15.2 to 16.3 per 100 eV of absorbed energy with an indication of a possible increase in G (Fe^{3+}) with increasing energy. However, with typical measurement uncertainties of $\pm$ 2% and differences of up to 5% for the same energy, no conclusion can be drawn until more accurate measurements are available.

A measurement of G (Fe^{3+}) has two components (a) the measurement of the number of ferric ions produced in the dosemeter and (b) the determination of the absorbed energy which produced the ions. In previous G (Fe^{3+}) measurements the two components have been treated as separate experimental measurements. The dosemeter solution has been irradiated and the ferric ion concentration determined from optical measurements in a spectrophotometer. The energy absorbed in the solution has been derived from measurements either with an ionization chamber or a disc calorimeter, or, if the dosemeter absorbed all the incident electrons, from the charge deposited in the solution and the electron energy. The division of the G (Fe^{3+}) measurement into two parts requires reproducibility of the electron radiation for both parts and introduces uncertainties in deriving the energy absorbed in the ferrous sulphate dosemeter from measurements using an ionization chamber or disc calorimeter.

The present technique combines both parts of the G (Fe^{3+}) measurement by using the ferrous sulphate solution both as a chemical dosemeter and simultaneously as a calorimetric medium.

Results of Project No. 1

Head of Project : B Owen

and scientific staff : W T Morris
J H Barrett

Title of Project : MEASUREMENT OF G (Fe^{3+}) FOR 2 MeV ELECTRONS

The calorimeter was designed to totally absorb within the solution the 2 MeV electron beam from a Van de Graaff generator. The calorimeter vessel was constructed from 1 mm thick silica with a 1 mg cm^{-2} polyethylene terephthalate film entry 'window'. The calorimeter contained about 260 g of ferrous sulphate solution and was continuously stirred by a silica paddle to circulate fresh solution into the irradiated volume behind the entry window and to distribute the heat uniformly throughout the calorimeter. The absorbed energy was measured by means of a thermistor in a silica tube dipping into the solution. The thermistor was in one arm of a d.c. Wheatstone bridge circuit and the system had a temperature sensitivity of about 10^{-5} °C. The thermistor was calibrated in terms of electrical energy by passing a measured current through a known value resistor in another silica tube immersed in the solution. The calorimeter vessel was contained in a double-jacketed, temperature-controlled air enclosure. The electron beam was collimated to about 10 mm in diameter at the calorimeter window, the window itself being about 40 mm in diameter, and a shutter controlled the irradiation period.

The dosemeter solutions were prepared using the same techniques as for the NPL chemical dosemeter service and the response of each batch of solution to cobalt-60 irradiation was checked. The calorimeter and glassware used to handle the solution were cleaned using both chemical and irradiative methods.

In use the calorimeter reached thermal equilibrium overnight and the bridge was then balanced. The out-of-balance voltage of the bridge was used as an indication of the changes in the calorimeter temperature. During the pre- and post-heating periods the bridge voltage was continuously recorded at known time intervals to establish the drift of the calorimeter temperature with time. To correct for the drifts, the data from the two periods were analysed by computer using linear least squares fits and extrapolated to the

mean time of heating. The difference between the two extrapolated values gave the corrected voltage change due either to electron irradiation or to electrical calibration heating. A typical irradiation was 4 k rad in 100 s resulting in a temperature rise of about 0.01°C.

After irradiation and calibration, samples of the calorimeter solution were taken and their optical absorption at 304 nm measured in a spectrophotometer to determine the ferric ion concentration. Four separate batches of ferrous sulphate solution have been used to make 26 measurements of G (Fe^{3+}) using four different calorimeters. The beam current was varied to give dose rates, averaged over the whole solution, between 5 and 50 rad s^{-1} and over this range the value of G (Fe^{3+}) decreased by about 1%. This decrease is attributed to oxygen depletion in the relatively small volume of solution actually absorbing the electron beam. The linear least squares extrapolation of the measured values to zero average dose rate gave G (Fe^{3+}) = 15.46 based on an exothermic correction of 15.0%. The statistical uncertainty on G (Fe^{3+}) at the 95% confidence level is $\pm$ 0.2% from the calorimetry and spectrophotometry. The exothermic correction arises from the heat-of-reaction during the irradiation of the ferrous sulphate dosemeter and was calculated from published heats-of-formation assuming the overall reaction of Jayson, Parsons and Swallow, Int. J. Radiat. Phys. Chem. 7, 363-370, 1975. The heat-of-reaction is exothermic contributing an additional 15.0% in relation to the kinetic energy of the electrons. In view of the size of this correction, work has begun to confirm it experimentally by measuring the heat evolved per unit energy absorbed for both the ferrous sulphate dosemeter and aerated, aqueous 0.4 mol dm^{-3} sulphuric acid. The initial results are in approximate agreement with calculation. However, the main systematic uncertainty in the measurement of G (Fe^{3+}) arises from the exothermic correction, estimated at present as $\pm$ 1%, and further work is necessary to confirm this correction and to reduce its uncertainty.

Contractant de la Commission : COMMISSARIAT A L'ENERGIE ATOMIQUE -

N° du Contrat : 145-75-1 BIO F

Chef du groupe de recherche : Dr. N. PARMENTIER,
Chef du Laboratoire de Dosimétrie Sanitaire. CENFAR.

Thème général du contrat : DOSIMETRIE DES PARTICULES LOURDES CHARGEES

Les études faites sur le faisceau d'hélions de 650 MeV de l'accélérateur SATURNE à SACLAY sont de deux aspects, dosimétrique et microdosimétrique. Dans les deux cas, l'interprétation des mesures nécessite l'utilisation d'une valeur $\overline{W}$, énergie correspondant à la création d'une paire d'ions.

I - MESURES DE $\overline{W}$ (M. CHEMTOB, LAVIGNE B., NGUYEN V.D.)

La valeur de $\overline{W}$ qui varie en fonction de l'énergie et de la nature de la particule est très mal connue pour les ions lourds. C'est pourquoi, au cours de 1975, nous avons commencé des mesures de W systématiques pour différents types d'ions lourds à différentes énergies (25 keV à 500 keV).

L'expérience est montée sur un accélérateur SAMES mis à notre disposition par un autre service. Elle consiste essentiellement à mesurer alternativement le nombre de particules entrant dans le volume gazeux et le courant d'ionisation produit par ces particules.

Deux gaz ont été étudiés : - l'argon
- le mélange gazeux de ROSSI.

Les particules chargées choisies pour l'instant sont H^+, He^+ et Ar^+. Il est certain qu'une étude plus systématique devra être menée pour les différents gaz (CO^2, Azote et Oxygène) composant le mélange de ROSSI ainsi que pour les ions C^+, N^+ et O^+ produits de réactions nucléaires des hélions.

Les premiers résultats montrent que $\overline{W}$ varie dans la gamme d'énergie étudiée plus vite pour les noyaux lourds que pour les protons ou les hélions : ces variations sont de 30 % pour les noyaux Ar^+ par exemple et 10 % pour les He^+. Il semble de plus, que les courbes de variation en fonction de l'énergie présentent des structures, en particulier dans l'argon pour les ions He^+, d'énergie comprise entre 80 et 100 keV.

Les résultats préliminaires feront l'objet d'une communication au "WORKSHOP" organisé du 19 au 21 mai au TJNO par l' "ENDIP Coordination Committee".

II - RESULTATS MICRODOSIMETRIQUES

Les mesures de microdosimétrie faites dans un faisceau d'hélions de 650 MeV, après absorption dans différentes épaisseurs d'eau, posent des problèmes d'interprétation qui ne sont pas dus uniquement à la valeur de $\overline{W}$ choisie. Pour mieux cerner ces problèmes, un essai a été fait pour comparer les résultats obtenus par le calcul à partir des spectres des hélions et les résultats expérimentaux.

1. Résultats obtenus à partir de mesures par compteur proportionnel

Rappelons que l'énergie des hélions est dégradée par un disque de cuivre possédant 9 secteurs, afin de réaliser en profondeur un plateau iso-effet de 5 cm.

- La taille moyenne d'événement $\overline{Y}_D$ déduite des spectres mesurés par compteur proportionnel est pratiquement constante dans les premiers 3,5 cm du plateau : elle varie de 5,99 à 6,18 keV.μm^{-1}.

- Elle varie par contre d'un facteur 2,4 dans les quinze derniers millimètres.

2. Essai de calcul de la taille moyenne d'événement

A l'aide d'un semi-conducteur épais de 5 mm au silicium travaillant en perte totale d'énergie, le spectre des hélions a été obtenu à 17,82 g.cm^{-2} (dernier pic du plateau iso-effet). Un calcul fait à partir de ce spectre en utilisant les données de l'ICRU donne une valeur moyenne de taille d'événement $\overline{Y}_D$ de 61,2 keV.μm^{-1} alors que la mesure directe par compteur donnait une valeur de 14,83 keV.μm^{-1}.

L'étude du spectre des tailles d'événements montre que les hélions contribuent pour 79 % à la valeur de $\overline{Y}_D$, les 21 % restants correspondent à des faibles tailles d'événement. Un calcul simple montre que la contribution des rayons δ provenant de la paroi du compteur ne représente que 1,3 % de la valeur moyenne de $\overline{Y}_D$. L'influence des particules de faible T.E.L. est donc négligeable. On voit apparaître que la difficulté expérimentale est due à la fois aux variations rapides du T.E.L. autour du pic et à la forme du compteur.

Si la forme sphérique du compteur parait plus séduisante parce que plus proche de la réalité biologique, et que son utilisation en début de plateau ne pose pas de problème de géométrie, il serait préférable en fin de parcours des hélions de mesurer leur spectre énergétique pour avoir une valeur plus précise de $\overline{Y}_D$.

Contractant de la Commission :

COMMISSARIAT A L'ENERGIE ATOMIQUE
Centre d'Etudes Nucléaires de Fontenay-aux-Roses

N° du contrat : 065-72-01-PSTC

Chef du groupe de recherche : G. SOUDAIN

Thème général du contrat :

Recherche des moyens les mieux appropriés à la dosimétrie des photons et des neutrons dans les champs mixtes.

Description générale succincte des travaux réalisés.

L'étude de la sensibilité des détecteurs RTL aux neutrons s'est poursuivie auprès du VAN de GRAAF de Bruyères-le-Chatel et de Cadarache.

Nous avons étudié le ^{7}LiF, le ^{nat}LiF et le $CaSO_4$ (Dy).

Nous avons mis au point une méthode qui a servi de référence pour la dosimétrie γ dans les champs mixtes. Il s'agit de l'utilisation d'émulsions photographiques placées dans des écrans de plomb après suppression de tout radiateur hydrogéné.

A partir de cette référence on a pu déterminer avec une assez bonne précision la sensibilité des divers matériaux RTL aux neutrons.

Enfin des études parallèles ont été effectuées sur l'alumine par Emission Exoélectronique. Elles ont montré l'apparition d'un phénomène de stabilisation, phénomène qui doit être étudié avant de progresser dans nos recherches.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques :

G.PORTAL - R.MEDIONI, M.PETEL

Titre du projet :

Dosimétrie des photons en présence de neutrons.

Description des résultats.

1°/- On a vérifié que l'émulsion photographique constitue un excellent dosimètre pour le rayonnement γ dans un champ mixte, dans les conditions suivantes :

a) suppression de tout radiateur hydrogéné,
b) utilisation d'un filtre de Pb destiné à supprimer les photons de faible énergie.
c) étalonnage de l'émulsion à l'aide d'un émetteur d'énergie comparable à celle des photons mesurés.

Les résultats de l'émulsion photographique ont été comparés à ceux d'un compteur G.M. construit selon les spécifications de WAGNER et HURST; on a obtenu une bonne correspondance pour les photons d'énergie comprise entre 1 et 4 MeV.

2°/- Dans le tableau ci-dessous nous avons regroupé les résultats concernant la mesure de la sensibilité des détecteurs aux neutrons de 6 énergies.

Celle-ci est exprimée en unité $R\text{-}rad^{-1}$, c'est-à-dire que les chiffres mentionnés donnent la réponse apparente d'un matériau donné, étalonné en exposition, pour une dose absorbée de neutrons unitaire.

Ces résultats s'ajoutent à ceux que nous avons publiés à la fin de l'année 1974, concernant les neutrons des sources de ^{252}Cf, Pu-Be, les neutrons de 14 MeV et les neutrons thermiques.

L'analyse de ces résultats montre que le sulfate de calcium est un des matériaux les moins sensibles aux neutrons.

TABLEAU

	FLi 7		FLi N		SO_4Ca		réaction utilisée	
	Alu	Pl	Alu	Pl	Alu	Pl		
250 keV	0.009	0.013	0.062	0.063	<	<	$Li^7(p,n)Be^7$	Cadarache
716 keV	0.012	0.016	0.017	0.031	0.0025	0.005	"	"
2,2 MeV	0.013	0.022	0.017	0.026	0.0045	0.015	$T(p,n)\ He^3$	"
3,5 MeV	0.013	0.042	0.013	0.04	0.038	0.078	$T(p,n)\ He^3$	"
3 MeV	x	0.022	x	0.022	x	0.019	$T(p,n)\ He^3$	B III
7 MeV	0.04	0.075	0.035	0.08	0.035	0.07	$D(d,n)\ He^3$	B III (cible gazeuse)

Sensibilité des matériaux RTL aux neutrons de diverses énergies - Unités exprimées en $R.rad^{-1}$

3°/- L'étude de l'émission exoélectronique de l'alumine a mis en évidence un important phénomène de stabilisation; dans les 4 premières heures suivant l'irradiation le signal d'émission d'électrons décroît de 50 p.cent environ. Nous avons étudié différents types d'alumines; tous ceux qui présentent ce phénomène sont remarquablement sensibles aux photons. Il s'agit probablement d'un phénomène de redistribution des porteurs.

Il a été décidé d'étudier cette anomalie avant de poursuivre nos études sur les caractéristiques de l'alumine.

COMITATO NAZIONALE PER L'ENERGIA NUCLEARE

LABORATORIO FISICA SANITARIA, BOLOGNA(Italy)

Contract No.: 065-72-1 PSTC
Leader of the Research Projects: G.Busuoli
General Subject of the Contract:

STUDIES ON NEW DETECTORS USEFUL FOR PERSONAL DOSIMETRY

Project No.1
Project Leader: G.Busuoli
Project Title:

APPLICABILITY OF TSEE DETECTORS TO PERSONAL DOSIMETRY

Experimental Results during 1975

As said in the previous report, during 1975 we have checked BeO uncoated to verify its reprodubility when used as exoelectron emitter. The results have been unsatisfactory and at beta doses of about 100 mrad from Sr-90 source the reproducibility was of the order of $\pm$ 25%.

During the year it was found that the workind conditions of the counter became modified; a new G.M. counter was set-up with the same diameter but with a greater length in order to avoid dead volumes near the collecting volume.

The experimental data obtained with this new apparatus have been completely unsatisfactory as the reproducibility was again of the order of 20-25% for ten readings on the same detector. The same results have been found either annealing the detectors at 600°C for 15 min after each reading, or by irradiating the detectors annealed simply by the read-out procedure. On the same detectors,tests on the response as a function of the energy have been performed,too. The BeO discs have been irradiated inside a simple plastic container. Due to the large variability of the responses connected to the low reproducibility, the data obtained for the energy response have no physical meaning.

Perhaps best results could be obtained with detectors coated with graphite,evaporated on their surface. Unfortunately it is not easy to obtain

samples with the same graphite layer; furthermore the deposition method is very time-consuming and it could not be used in a simple way on a routine basis.

We can conclude that TSEE method seems not applicable in a simple way to personal dosimetry. On the other hand, the interest on it seems to be largely slowed-down and many criticism are expressed also by people working in foreign laboratories. Probably, due to its peculiar characteristics, TSEE shall be used to perform special dosimetric measurements under strictly controlled conditions.

Project No.2
Project Leader: A.Cavallini
Project Title:

CRITICALITY ACCIDENT DOSIMETRY BY PLASTIC TRACK DETECTORS

Experimental Results During 1975

In 1975 the tests to verify the utilization of plastic detectors in fast neutron personal dosimetry by the albedo technique have continued.

The prototype dosimeter is made by Al coated with LiF as (n, α) converter, by cellulose nitrate, by Cd, again by cellulose nitrate and, finally, by Al coated by LiF. This set up allows to detect both the neutrons backscattered by human body (second plastic detector) and the direct neutrons (first plastic detector). The Cd screen, placed between the two cellulose nitrate foils, allows to separate the two neutronic components.

A big difficulty came from the deposition of the LiF layer on the Al support in order to obtain converters of equal efficiency. It was thus necessary a calibration of the converters sensitivity by exposure to a radioactive source of thermal neutrons and by keeping them in contract with a plastic detector

The prototype dosimeters have been exposed to several dose values and to 5 neutron energies in the range 0.2 - 14 MeV. It has been found that with the dosimetric system described it is not possible to evaluate doses down to 500 mrem.

As concerns the energy dependence of the response, it has been shown that the ratio of the responses of the two plastic detectors (i.e. the ratio of reflected to direct neutrons) is a function of the energy; hence it seems possible to correct the dosimeter response and avoid this kind of systematic error.

Different tests have dealt with the behaviour of the chemical etching as a function of the induced damage in the plastic exposed to high gamma doses. These tests have been performed in order to try to rise the detection sensitivity of the plastics and therefore to lower their detection threshold.

Contractant de la Commission : DSN/SESTR-CEA (FRANCE)

N° du contrat : EUR 65 - 72 - 1 PSTC

Chef du Groupe de Recherche : M. Michel BRICKA

Thème général du contrat :

Développement d'appareils de mesure des fluences et doses de neutrons au niveau de l'ambiance.

Description générale des travaux réalisés :

Deux nouvelles campagnes de mesure faites auprès du Van de Graaff de 5 MeV du CEN/Cadarache n'ont pas permis d'expliquer la divergence constatée, pour les grands diamètres de sphères entre valeurs mesurées et valeurs calculées.

La réponse intégrale, sur une source américium - beryllium, peut être calculée pour la courbe de réponse mesurée et la courbe de réponse calculée. Les résultats comparés avec celui de la mesure directe conduisent à rechercher de façon empirique une courbe de réponse intermédiaire.

Au niveau des énergies épicadmiques des résultats encourageants ont été obtenus, mais pour obtenir une bonne précision, il est nécessaire d'éliminer, autant que faire se peut, les neutrons thermiques. L'équipement du bloc SIGMA avec des écrans de boral et de gadolinium est en cours.

Le spectromètre à intégrateurs passifs d'indium, intéressant pour les mesures dans les Centrales électronucléaires, a été mis en forme. Il serait nécessaire d'améliorer sa sensibilité. Pour utilisation dans les remmètres, un compteur à ^{3}He de plus grand volume (diamètre 25 mm) a été développé.

Diverses méthodes pour l'établissement de formules linéaires ont été essayées. Il parait souhaitable de définir une pondération qui tienne compte de la répartition des doses en fonction de l'énergie, une bonne précision n'étant pas nécessaire dans les bandes ou les doses sont, relativement, faibles. Une version nouvelle du programme de calcul - - SPEC 2000 - a été établie. Elle permet, dans l'application de la méthode des spectres modèles, d'effectuer certains calculs concernant la précision et comporte un important bloc de données.

Enfin, des études préliminaires ont été effectuées concernant la source de référence prévue pour l'intercalibration des ensembles de sphères utilisés par les différents Services de Radioprotection du C.E.A. La source d'americium-beryllium a été retenue mais il apparait difficile d'obtenir des données cohérentes sur le spectre de ce type de sources.

Résultats du projet N° 1

Chef du projet : M. Michel MOURGUES

Titre du projet : Développement d'appareils de mesure des fluences et doses de neutron.

Description des résultats :

Courbes de réponse

Deux nouvelles campagnes de mesures ont été faites pour tenter d'expliquer les divergences entre valeurs mesurées et valeurs calculées, pour les énergies supérieures à 1 MeV. Cette divergence dépasse 20 % pour la sphère de 12" à l'énergie de 7 MeV.

Chaque détermination comporte une mesure suivie d'une deuxième mesure avec une barre d'ombre donnant l'évaluation du diffusé qui est ensuite soustrait.

On a donc vérifié l'efficacité de la barre d'ombre en la prolongeant par un cylindre de laiton de 20 cm. Les mesures faites dans ces conditions recoupent parfaitement les mesures antérieures.

On a également évalué le diffusé par une série de mesures à différentes distances de la cible de l'accélérateur. La fluence directe suivant une loi en $1/d^2$, on peut par ce procédé obtenir la loi de variation du diffusé. Ces mesures ont confirmé la validité des résultats donnés par la barre d'ombre.

Il est donc nécessaire de remettre en cause :
- soit l'étalonnage du moniteur du Van de Graaff,
- soit la nature monoénergétique du rayonnement de l'accélérateur.

Des vérifications dans ce domaine sont malheureusement exclues, le Van de Graaff du CEN/Cadarache cessant son activité en début de 1976.

La figure 1 présente pour les sphères de 8", 10" et 12" les deux séries de valeurs - mesurées et calculées - pour les énergies supérieures à 100 KeV.

A partir de ces courbes et du spectre de la source étalon d'americium-beryllium déterminé par M. BENEZECH (scintillateur liquide), il est possible de calculer la réponse intégrale en nombre d'impulsions par neutron-cm^{-2}.

$$R_c = \int_0^{E_{max}} R(E) \cdot \Phi(E) \, dE$$

Le tableau ci-dessous donne le résultat de ce calcul pour les deux courbes de réponse ainsi que les valeurs obtenues par mesure de la source Am-Be.

Diamètre de sphère	8"	10"	12"
Courbe de réponse calculée	0,366	0,368	0,315
Courbe de réponse mesurée	0,309	0,298	0,262
Mesure directe de la source	0,349	0,334	0,298

Les valeurs obtenues à partir des courbes de réponse calculées sont très proches de celles de la mesure directe, mais la proportionnalité entre les trois diamètres n'est pas respectée, le rendement de la 10" étant supérieur à celui de la 8", contrairement aux données de la mesure directe.

Avec les courbes de réponse mesurées, la proportionnalité est parfaitement respectée, mais toutes les valeurs calculées sont inférieures de 12 % aux données de la mesure directe. Il a donc été décidé de rechercher de façon empirique, en tenant compte des résultats précédents, des courbes de réponse compatibles avec la mesure directe.

Pour les énergies épicadmiques, les essais avec des fenêtres ont été poursuivis. Il est apparu que la fluence thermique très élevée du bloc SIGMA nuisait à la précision des mesures. La face avant du bloc a donc été équipée d'un écran de Boral dans lequel est ménagé un trou de 140 mm qui constitue la source de neutrons thermiques et épicadmiques.

Un filtrage thermique par une solution de nitrate de Gadolinium a été essayé, mais la présence d'eau s'est révélée néfaste. Des plaques de Gadolinium ont donc été commandées. On espère avec ce filtrage pouvoir créer deux "fenêtres" :

- Cadmium 17/100 - Cadmium 14/10 : énergie moyenne 0,4 eV
- Cadmium 14/10 - Boral : énergie moyenne 5 eV

Ces fenêtres couvriraient convenablement la zone ou se trouvent les pics des courbes de réponse des sphères de petits diamètres - 2", 2,5" et 3"-.

Spectromètre intégrateur passif

Pour les besoins de mesures en Centrales Electronucléaire, le système de sphères à détecteurs activables a été développé. Un montage à quatre sphères - 2,5", 2,5" sous cadmium, 4,2" sous cadmium, 10" sous cadmium - (figure 2) a été réalisé. Il est équipé d'ensembles de 5 détecteurs d'indium qui sont mesurés simultanément sur compteur Amperex 18536. Du point de vue opérationnel, ce matériel est satisfaisant, mais sa sensibilité faible, entraine des durées d'irradiation et de comptage relativement longues. Au niveau de 10 mrem.h^{-1}, il faut 1 heure d'irradiation et 4 x 20 minutes de comptage. Il serait donc souhaitable d'augmenter d'un facteur 10, au moins, la sensibilité.

Compteur ^{3}He de 25 mm

Le compteur 0,5 NH 1/1 développé antérieurement s'étant avéré trop peu sensible pour l'utilisation dans un compteur rem, de nouveaux prototypes de compteurs à hélium de diamètre 25 mm ont été réalisés par L.M.T. et sont actuellement en cours d'essais.

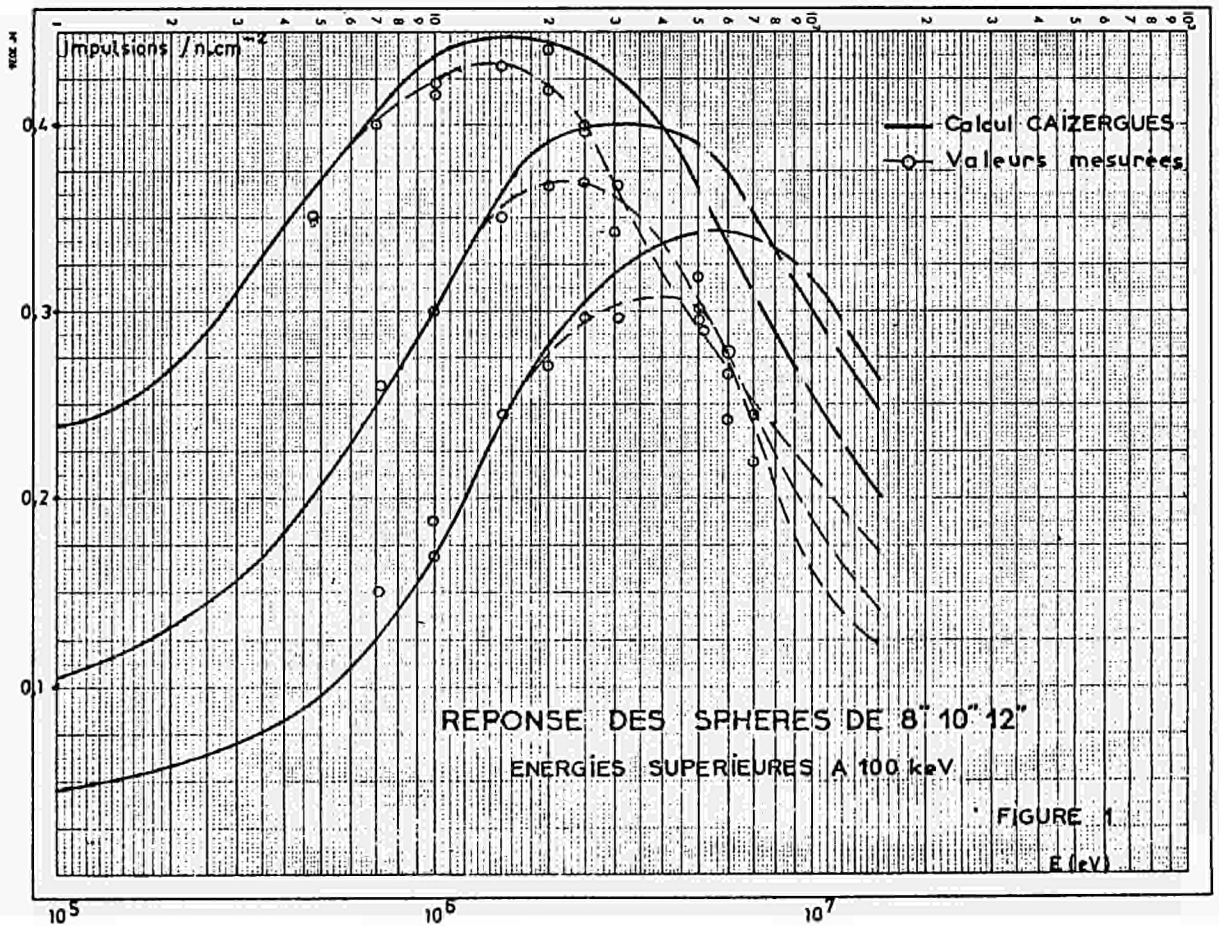

Impulsions / n.cm⁻²
0,4
0,3
0,2
0,1
Calcul CAIZERGUES
Valeurs mesurées
REPONSE DES SPHERES DE 8" 10" 12"
ENERGIES SUPERIEURES A 100 keV
E (eV)
10⁵
10⁶
10⁷

FIGURE 1

SPECTROMETRE INTEGRATEUR NEUTRONS

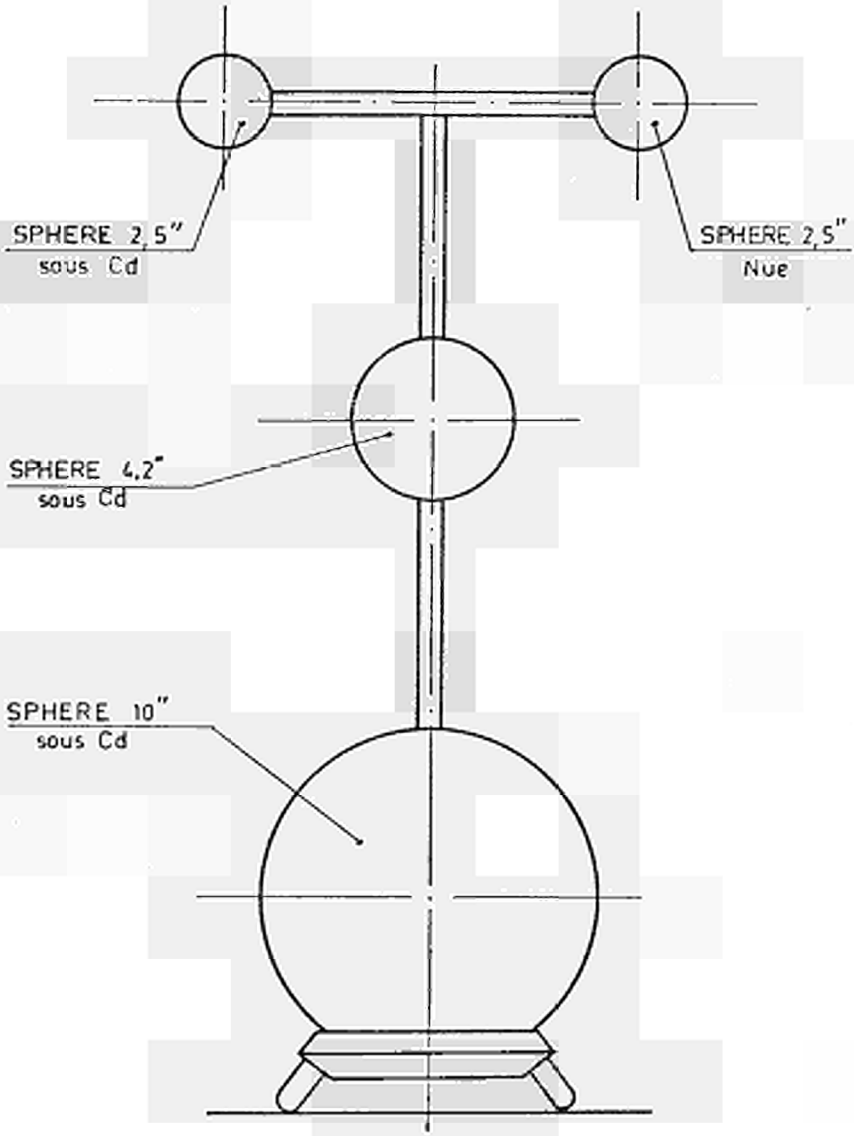


FIGURE 2

Résultat du projet N°. 2

Chef du projet : M. Michel BUXEROLLE

Titre du projet : Développement de formules et programmes de calcul des fluences, doses et spectres de neutrons.

Description des résultats :

Formulation linéaire

Diverses méthodes ont été essayées pour obtenir la formulation linéaire des fluences et doses pour un système à sphères multiples :

- soit à partir de la fonction log-normale qui représente valablement les taux de comptage en fonction du logarithme du diamètre,
- soit en recherchant des combinaisons linéaires des courbes de réponse des sphères susceptibles de représenter :
  - une horizontale pour les mesures de fluences :

$$\Sigma \alpha i \quad Ri\ (E) \quad \equiv \quad \text{Constante}$$

  - la courbe de dose équivalente, pour les mesures de dose :

$$\Sigma \beta i \quad Ri\ (E) \quad \equiv \quad D\ (E)$$

Il apparait qu'une pondération est nécessaire pour obtenir le maximum de précision dans les bandes d'énergies ou les doses sont relativement les plus importantes, c'est-à-dire, en thermiques et au-dessus de 100 KeV.

Programme de calcul

Une version nouvelle du programme de calcul, le programme SPEC 2000 a été établie. Toujours basé sur la méthode des spectres modèles, ce programme est applicable à tous les types de détecteurs dont la courbe de réponse en fonction de l'énergie est connue. Il comporte un important bloc de données, ce qui diminue considérablement le nombre de cartes à manipuler et permet de tenir compte des précisions d'étalonnage et de mesure pour effectuer le calcul de la précision des résultats et du domaine de validité du spectre correspondant.

Comme les programmes précédents, il est évidemment à même de donner les formules linéaires qui permettent, à partir des données de mesure, le calcul des fluences et doses sans recourrir à l'ordinateur.

Source de référence

En vue de l'étude d'un système de référence pour l'étalonnage des fluencemètres et dosimètres neutrons, prévue pour 1976, il a été décidé, faute d'un faisceau de neutrons monoénergétiques calibré, d'adopter une source americium-beryllium comme source de référence.

L'étude de la bibliographie faite par M. BUXEROLLE révèle des divergences sur la forme du spectre pour les énergies inférieures à 1,5 MeV.

Il est donc prévu de demander à M. BENEZECH, qui dispose d'un ensemble de compteurs proportionnel à protons de recul et d'un scintillateur liquide, d'établir le spectre de la source pour la bande d'énergies 10 KeV - 10 MeV.

Le débit de fluence de cette source, par contre, mesuré par la méthode du bain de manganèse au LMRI, est connue avec une bonne précision.

Kernforschungsanlage Jülich GmbH, Zentralabteilung Strahlenschutz
D 517 Jülich

Contract No. 065-72-1 PSTC

M. Heinzelmann

Neutron dosimetry with moderator spheres

This year the research of the previous year on a light rem-counter was extended to an energy independent personal dosimeter on the same principles. It could be shown that it was possible to build a personal dosimeter for neutrons with a ^{3}He-counting tube and a thin moderator.

A Monte-Carlo-Program for the calculation of neutron moderation in spherical layered geometry with cylindrical measuring devices was written.

Further the influence of homogeneously distributed strongly absorbing material like Boron in the moderating ball on the sensitivity of the dosimeter device was investigated. It was shown, that there is no substantial improvement of the energy-dependency of the dosimeters.

Results of the project:

Neutron dosimetry with moderator spheres

F. Rohloff and M. Heinzelmann

a) An albedo dosimeter with ^{3}He-counter

In the previous year a rem-counter of small weight had been proposed. This device has been modified for use as a personal dosimeter. In albedo-dosimetry the body of the user of the dosimeter serves as an additional moderator for the incident neutrons and alters the reading of the dosimeter. Therefore the moderator of a personal dosimeter may be leightened considerably. We have used a moderator thickness of 0,5" and two different ^{3}He-counting tubes of type 0,5 NH 1/1 K from L.M.T. and type SP9 from 20th Century. Experiments with two different counting tubes show that the results do not differ with the type of the tubes.

The dosimeter has been irradiated on a Thorax-Phantom with neutrons from different sources. It has been proved that with an appropriate discriminating threshold the reading of the dosimeter is very well independent of neutron energy.

The smaller counting tube has a sensitivity of 1,4 pulses per mrem. The γ-sensitivity grows with larger neutron dose rate. For neutrons of an Am-Be-source and a neutron dose rate of 300 $^{mrem}/h$ in the field of a γ-dose rate of 5 $^{r}/h$ the sensitivity for the γ-radiation of ^{60}Co is less than 0,5 % of the sensitivity for neutrons.

Our device requires a very reliable electronic. A variation of the discriminator threshold of one percent alters the neutron sensitivity by 6,6 %. An alteration of the detector voltage of 1 % alters the neutron sensitivity by 7,5 %.

The experiments show that the proposed method is useful for the personal dosimetry of neutrons. To be applicable in field praxis, we have to develop a reliable and leight electronic, so that the dosimeter device can be conveniently worn by a person to be monitored. The discriminator and amplifier are already constructed. The other aimes are developed.

b) A Monte-Carlo-Programm for moderating spheres with cylindrical elements

This year the work on a Monte-Carlo-Program was finished. This program calculates the transport of neutrons in a moderator consisting of spherical layers of different material and takes into account cylindrical elements as light pipes and detectors. Figure 1 gives a general survey of the program-structure. Main elements are subprogram INIT for the initialisation of free paths, a subprogram ORT for the calculation of positional parameters within the sphere, and a subprogram TEST, ZYLORT for positional parameters within the cylinders. There are subprograms ABSORB and ABSOR for absorption-processes or variation of weights, subprogram ELAS and ANITRO for elastical, inelastical and anisotropical scattering. GEMEIN is a pilot-subprogram for optimal calling of other subprograms.

c) Calculations with borated spheres

We have investigated the influence of strong absorbers like Boron within a polyethylene moderator on the sensitivity of a Bonner sphere by calculation*. As expected, the sensitivity of e.g. a 10" ball goes down in the mean by a factor 1,8 for 0,1 % of Boron and by a factor 9,0 for 1 % of Boron in percents by weight of hydrogen. But there is no significant variation of the shape of the sensitivity as a function of energy for the 10" sphere. The results of the calculations with a 5" sphere are given in Fig. 2. The loss of sensitivity for 1 % of Boron is greater by a factor of two at the energy of 10 eV compared with that at 10^6 eV. For 10 % of Boron the loss of sensitivity goes down stronger at lower energy, but the general loss of sensitivity is unconveniently high. Thus it was shown that an improvement of dosimeters by a borating technique, is not to be expected.

*) This has been proposed by Mr. Bricka, Cadarache.

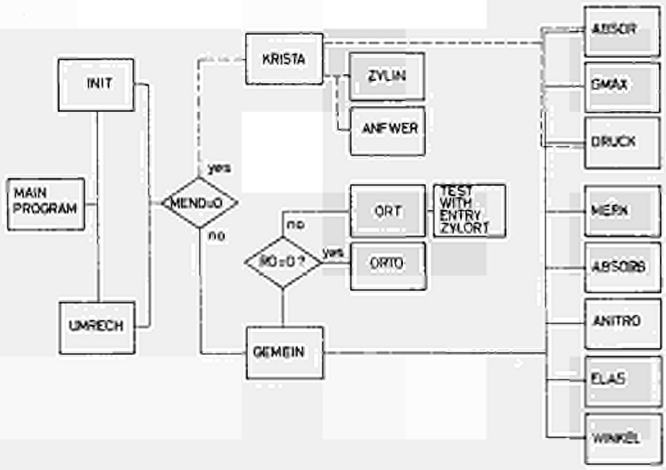


Fig. 1: Survey of program interaction

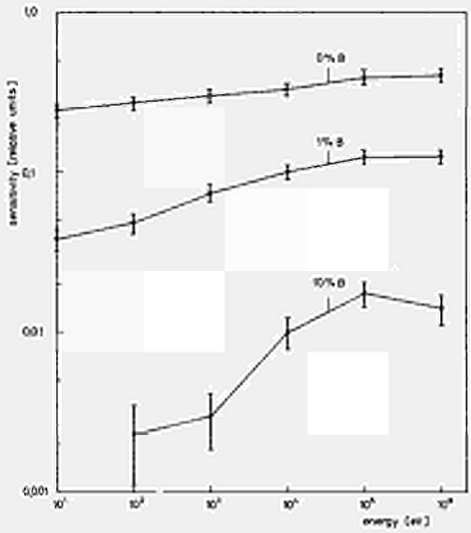


Fig. 2: Results of a Monte-Carlo-calculation of the sensitivity of a 5" sphere of borated polyethylene.
Given are percents in weight.

Publications:

M. Heinzelmann, H. Schüren

Untersuchungen für ein energieunabhängiges Neutronen-Personendosimeter
9. Jahrestagung des Fachverbandes für Strahlenschutz, Alpbach, Tirol, 6. - 8.10.1975

Apply for a patent:

M. Heinzelmann, F. Rohloff, H. Schüren

Gerät zur Bestimmung der Äquivalentdosis von Neutronen

Angemeldet:	BE	160712	(6.10.1975)
	EI	2202/75	(8.10.1975)
	FR	75 30 512	(6.10.1975)
	GB	40 866/75	(6.10.1975)
	IT	28 023 A/75	(8.10.1975)
	LU	-	(6.10.1975)
	NL	75.11548	(1.10.1975)
	US	617,397	(29.9.1975)

GESELLSCHAFT FOR STRAHLEN- UND UMWELTFORSCHUNG MBH, MONCHEN
Institut für Strahlenschutz, Neuherberg

Vertrag Nr.: 065 PSTC
Leiter der Forschungsgruppe:
Dr. G.Burger, Prof.Dr.F.Wachsmann

Allgemeines Thema des Vertrages:
Personendosimetrie und Kalibriertechnik im Neutronenstrahlenschutz

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The main topics of the neutron dosimetry group are investigations on
- practical aspects of neutron personel dosimetry and beam dosimetry,
- development and installation of generalized codes for radiation transport calculations,
- development and installation of sources, arrangements and methods for calibration purposes in the field of radiation protection monitoring and beam dosimetry.

24 men-months are planned for the contract, 12 men-months were used for neutron spectrometry, the rest split into activities of albedo dosimeter calculations and calibrations with monoenergetic neutrons at the 3 MeV-Van De Graaff accelerator of the GSF and of neutron background calculations.

References:

Burger, G., J.David und H.Schraube
Die speziellen Probleme der Neutronen-Personendosimetrie
9.Jahrestagung des Fachverbandes für Strahlenschutz e.V.
Alpbach. Tirol, 6.10.1975

Ergebnisse des Projekts

Leiter des Projekts und wissenschaftliche Mitarbeiter:

H.Schraube, G.Burger, F.Grünauer, K.Kolbe

Titel des Projekts: Personel Dosimetry and Calibration Techniques in Neutron Radiation Protection

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1. Neutron spectrometry

The investigations were mainly concerned with the determination of the response-functions for small scintillation probes with a relatively long lightpipe. A code (EFKØ) was developed, allowing the calculation of the light transfer from the scintillator to the multiplier. Fig.1 shows the relative light intensity, emitted from the scintillator window surface to the lightpipe (BASINT), and the relative intensities arriving at the PM-cathode for a lightpipe with and without reflecting coat.

2. Personnel dosimetry

The albedo-iontrack monitor combination was further investigated. The idea is, to use the albedo monitor only for most of the occupationally exposed persons and to add the Np-fission track detector as an operational monitor for a limited number of easily controllable workers.

We continued some experimental investigations of the albedo dosimeter with respect to parameters which may influence the response functions of the TLD pairs within the device, such as positioning within the moderator, air gap to the phantom etc. This results are not yet complete. Fig.2 shows some results of the directional phantom response for the standard device.

3. Radiation transport calculations

3.1 Study of scatter background

It is of principal interest for calibration purposes to know the neutron scatter background at an irradiation facility. It may be experimentally determined by shadow cone measurements with a Long-Counter or appropriate analysis of distance law measurements.

We investigated to what extend well established transport codes applied to simplified geometries yield reasonable results. We used the codes ANSIN and DØT.

In detail some parameters, as source distance from the wall and floor, height and tickness of the additional shielding, as well as the influence of the roof were studied. A report on the results is in preparation.

3.2 Albedo calculations

The adaption and use of the code DØT for adjoint transport problems, as peported last year, was not correct. For the optimization of albedo monitors and better understanding of the response of each type of personnel monitors, the code was therefore applied to a cylindrical phantom in the direct mode and the spectral albedo fluence calculated around a phantom at several surface distances.

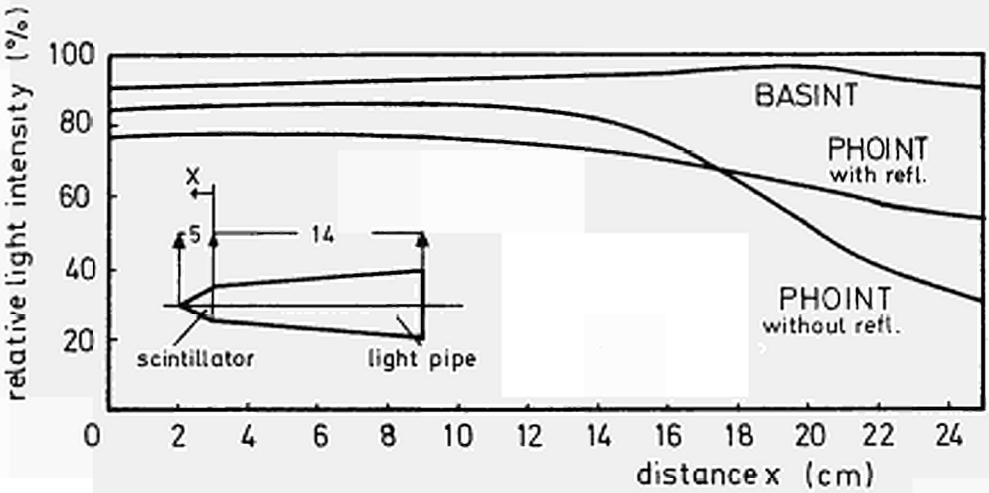

Fig.1 Relative light intensity for the shown scintillator and light pipe geometry

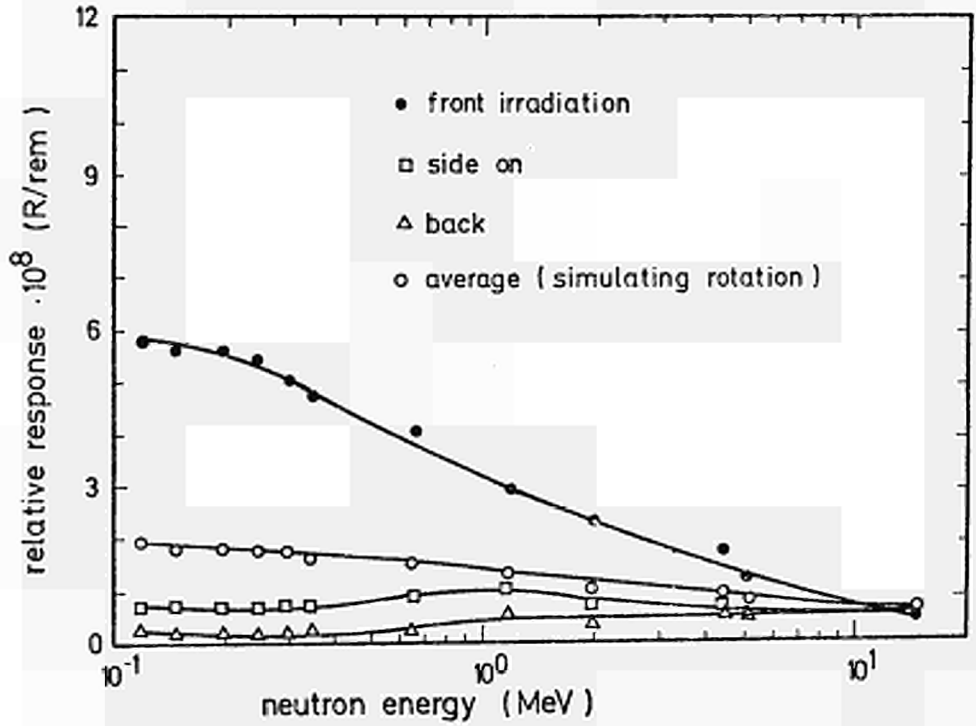

Fig.2 Relative response of the standard type albedo dosimeter around a phantom.

Contractant de la commission : Centre de Physique Atomique
118, route de Narbonne
31077 TOULOUSE CEDEX

N° du contrat : 069 73 1 PST F

Chef du groupe de recherche : D. BLANC

Thème général du contrat : Dosimétrie par mesure d'effets optiques et électriques dans les verres phosphates.

Description générale :

Pour suivre l'étude des propriétés électriques de verres non irradiés, nous avons étudié les effets du rayonnement gamma de sources de ^{60}Co en fonction de divers facteurs, champ électrique, débit de dose, température en particulier.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques : J. BARTHE, L. COMMANAY, J. CASANOVAS.

Titre du projet : Dosimétrie par conduction dans les verres aux phosphates.

DESCRIPTION DES RESULTATS.

La cellule de détection. Elle comporte le disque de verre métaphosphate maintenu entre deux plaques de téflon où sont fixées les connexions électriques. Les électrodes en or ou aluminium sont réalisées par métallisation sous vide, les contacts sont pris à la laque d'argent.

Résultats expérimentaux. Nous avons étudié le courant induit en fonction :

- du champ électrique (intensités 2.10^5 V/cm)
- de débits de dose inférieurs à 2600 rads/h dans l'air.
- à différentes températures (-15°C à +80°C).

Les caractéristiques $i_i(E)$ ont la même allure que celles obtenues pour les diélectriques liquides : il n'y a pas de courant de saturation. Le courant d'ionisation continue de croître pour des champs électriques supérieurs à ceux pour des champs électriques supérieurs à ceux pour lesquels on obtient une saturation dans le cas des gaz. Ce palier, de pente comprise entre 0,1 à 0,2 pA/cm.V, est suivi aux forts champs électriques ($> 6.10^4$ V/cm) d'une avalanche de porteurs de charges.

Les variations du courant induit avec le débit de dose, calculé dans l'air à l'emplacement de la cellule, est représentable par la relation :

$$i_i = K \left(\frac{dD}{dt} \right)^{\Delta}$$

La valeur de l'exposant Δ est voisine de 0,5 quel que soit le champ électrique et la température pour les échantillons sans argent ($Ba(PO_3)_2 + Na\,PO_3$).

Par contre, pour les compositions contenant Ag PO_3 (8 et 12 %), on observe des valeurs différentes (0, 5 ⟶ 1).

Tableau 1

	Verre à 0 % $AgPO_3$				Verre à 8 % $AgPO_3$				Verre à 12% $AGPO_3$
T °C / E(V/cm)	20°	10°	0°	-13, 5°	22°	10°	0°	-13,5°	20°
10^4	0, 546	0, 466	0, 493	0, 494	0, 438	0, 495	0, 489	0, 477	0, 486
2.10^4	0, 445	0, 500	0, 517	0, 480	0, 515	0, 476	0, 514	0, 440	0, 524
4.10^4	0, 445	0, 573	0, 576	0, 515	0, 522	0, 528	0,536	0, 499	0, 539
6.10^4	0, 569	0, 527	0, 409	0, 420	0, 586			0, 435	0, 631
8.10^4	0, 552	0, 484			0, 630				0, 701
10^5	0, 544	0, 553							0, 821

Valeurs expérimentales de l'exposant Δ dans la relation $I_i = K(\frac{dD}{dt})$

Dans la représentation log i_i f(1/T), nous avons étudié les coefficients de température ou énergie d'activation équivalente des porteurs de charges de la conductivité induite suivant les débits de doses et l'intensité du champ électrique.

Tableau 2

	Verre à % $AgPO_3$		Verre à 8 % $AgPO_3$	
dD/dt rads/h / E(V/cm)	870	2610	870	2610
10^4	48 MeV	30 MeV	1, 3 MeV	1, 3 MeV
2.10^4	26 MeV	40 MeV	10 MeV	9 MeV
4.10^4	70 MeV	74 MeV	13 MeV	3 MeV
6.10^4	100 MeV	60 MeV	80 MeV	80 MeV
8.10^4	300 MeV	260 MeV	300 MeV	200 MeV

Valeurs expérimentales du coefficient de température U_i du courant induit : $i_i = K' \exp(-\frac{Ui}{kT})$.

Contractor: United Kingdom Atomic Energy Authority,
Atomic Energy Research Establishment, Harwell

Contract No.: 074-74-1 PSTUK

Head of research team: D.H. Peirson

General subject of contract: PASSIVE DOSIMETRY

This contract included two projects which are concerned with providing personnel dosimetry systems, particularly for mixed fields of neutrons and gamma radiation, and installed systems for passive spectrometry of neutrons.

Project 1. Passive detectors for neutron dosimetry and spectrometry

Activation detectors and fission foils used with dielectric track detectors are being studied for measuring neutron radiation from pulsed sources and at high dose rates. These detectors are being used as part of a passive neutron spectrometer and for personnel dosimetry.

Project 2. Dosimetry in mixed radiation fields

This project is concerned with improving methods of passive dosimetry for photon, neutron and ionising radiations. Thermoluminescent materials were chosen for the study as they have applications in beta and gamma-ray dosimetry (^{7}LiF) and albedo neutron dosimetry (^{6}LiF). The objective is to determine the limitations of the thermoluminescent material and to provide calibration data for the wide range of dosimeters at present in use. Theoretical calculations of the dose to the skin from activity deposited on the skin surface have been made to improve derived working limits for a wide range of isotopes.

Results of Project No. 1

Head of Project and scientific staff: J.A.B. Gibson
P.D. Holt
K.G. Harrison
S.J. Boot

Title of Project: PASSIVE DETECTORS FOR NEUTRON DOSIMETRY AND SPECTROMETRY

Development of a personal neutron dosimeter based upon ^{237}Np fission

The ^{237}Np fission foil (4 mg) has now been chosen as the main detector in the personnel dosimetry system designed for a fuel processing plant (see project 2). The response of the detector has been measured both in free-air and on a phantom using monoenergetic neutrons of energies from 0.05 to 1.7 MeV. Neutron sources such as ^{252}Cf, for fission neutrons, Pu-Be for (α,n) neutrons have also been used. A Sb-Be source produced neutrons at about 25 keV and by using a boron loaded moderator it was possible to obtain neutrons with a mean energy of 0.5 keV. These calibrations confirmed the prediction that the response on a phantom exceeds that in free air due to albedo neutrons of energies of thermal and intermediate energies. These are detected due to a significant response of the ^{237}Np below the 0.5 MeV threshold.

A suitable window material has been found to prevent access to the Np whilst still allowing the fission fragments through and by incorporating a shield behind the source the dose to the wearer can be reduced to a low level. The dosimeter will be fixed to a belt incorporating other dosimeters and the sensitivity is such that with the window 1 track is equivalent to 5 mrem. The tracks will be counted with a semi-automatic spark counter and the whole system is to be installed in a fuel processing plant.

Neutron spectrometry using activation and fission detectors and moderating spheres

Previously we have described the threshold detector system based upon fission foils of uranium and neptunium and the activation of gold and sulphur. We have also reviewed the whoel range of materials to look for other suitable threshold detectors. Our conclusion at the end of 1974 was

that the Bonner sphere system was the best choice to obtain information on the intermediate energy region. We therefore put in hand manufacture of 8 spheres.

The 8 spheres chosen have diameters of 2, 3, 4, 5, 6, 7, 8 and 10 inches which, with a bare detector, gives 9 results for any spectrum. The detector is a crystal of Li I (dia 8 mm, thickness 2 mm). In preparing for this work we found that two different sets of response functions were available: those calculated by Monte Carlo techniques by Awschalom and those obtained experimentally by Bramblett, Ewing and Bonner. Thus our early work has been to establish the response of our system.

We have used a ^{252}Cf source both bare and in polythene absorbers of radii 10, 20 and 40 nm for which Cross & Ing have calculated spectra. We also used the thermal column of Gleep which has a cadmium ratio of 0.019. The present interpretation is based upon a model with 5 parameters, i.e. thermal, intermediate and fast flux, the power p of E^{-P} for the intermediate energy neutrons and finally the mean energy of the fast maxwellian. The experimental responses so obtained have then been used to measure the background spectrum near a source store and the spectrum near to the DIDO heavy-water reactor. Other measurements have been made on neutrons produced by high energy protons in which the mean energy was 1 MeV but there was a significant flux above 20 MeV.

We are not happy with the response data so far obtained and would like to confirm which of the responses reported earlier by other workers is correct. The response is always influenced by the light pipe and photomultiplier of the detector and this can give differences compared with theory from 4% for the largest sphere to 50% for the smaller spheres. Also, using LiI, we have found that the use of cadmium as a shield gives rise to high energy γ rays ($\sim$ 7 MeV) which give an erroneous reading. Therefore we have not used cadmium in our experiments.

For the future we want to improve the response function and to produce an interpretation in terms of a histogram over about 7 or 8 energy bands. We also want to obtain the response using TLD's as the detector and to incorporate the Bonner spheres with the threshold detectors to produce a complete neutron spectrometry system which can be used for a

wide range of neutron spectra and dose rates. This spectrometry system will be used in the interpretation of the ^{237}Np personnel dosimeters.

References

Awschalom, M.
Use of the multisphere neutron detector for dosimetry of mixed radiation fields.
Neutron Monitoring: Proc. Symp. IAEA Vienna p289, 1966.

Bramblett, R.L., Ewing, R.I., Bonner, T.W.
A new type of neutron spectrometer
Nucl. Instrum. & Methods 9, 1 (1960).

Cross, W.G., Ing, H.
Prediction of fast neutron spectra in criticality accidents
Neutron Monitoring for Radiation Protection Purposes, IAEA Vienna 1973.

Results of Project No. 2

Head of Project and scientific staff: J.A.B. Gibson
M. Marshall
J.A. Douglas
T. Budd

Title of Project: DOSIMETRY IN MIXED RADIATION FIELDS

Dosimetry system for fuel reprocessing plants

This work can be considered as two interlocking parts, an integrated neutron dosimetry system and an integrated personal dosimetry system. The former covers the whole spectrum of neutron dosimeters, personnel dosimeters, survey instruments, installed instruments and measurements of neutron spectra. Methods being developed are outlined below.

The personal dosimetry system incorporates all those dosimeters which require to be worn by personnel. A belt has been designed to incorporate a ^{237}Np fission foil, 2 albedo dosimeters (front and back), 4 criticality lockets, a film badge, a personal air sampler, pump and batteries, and a pocket neutron dosimeter. Particular attention is being paid to the method of fixing the dosimeters on the belt, and the comfort of the wearer.

To evaluate the responses of the personnel and pocket neutron dosimeters three major experiments have been undertaken (October 1974, February 1975 and September 1975) using monoenergetic neutrons of 50 keV to 1.7 MeV from a Van de Graaff accelerator. This is an essentially scatter free facility 5 m from the floor of an ex aircraft hangar. Other experiments have involved thermal neutrons from a reactor and various neutron sources, particularly an antimony beryllium source. Theoretical studies have been made where possible to check experimental results and to enable the data to be extrapolated to other energies. Details on the albedo dosimeter are given below and the fission foil system has been described in Project 1.

In the course of these experiments various ancilliary experiments various ancilliary experiments and comparisons were performed. Our de Pangher long counter, has been compared with the Harwell long counter (Nuclear Physics Division). Good agreement was obtained. A neutron monitor of the Andersson-Braun type (AERE type 0075) has been calibrated.

Discrepancies between this and earlier calibrations are mainly due to the use of different flux-to-rem conversion factors (previously as in ICRP 4, now as in ICRP 21). These results are still being analysed.

The neutron spectra used in the IBIS experiment were determined using the time of flight technique. As expected the higher energy spectra are monoenergetic but at 100 keV and below the spectra become rather broad with a low energy 'tail'. These spectra will be taken into account in the final calculations.

The albedo dosimeter for neutrons

An albedo dosimeter detects neutrons which have been thermalised in the body. It has a high response for intermediate neutrons and a reduced response for fast neutrons. The thermal neutron detector presently used is a lithium fluoride TLD chip enriched in ^{6}Li (LiF-6). A correction for the photon response is obtained from a similar TLD highly depleted in ^{6}Li (LiF-7).

In the initial experiments various forms of albedo dosimeter were compared. This year more detailed measurements were made on the energy and angular response of the Harvey dosimeter. The experimental results for energy dependence agree with those expected from theoretical albedo data. A report on this work is being written.

Response of CaF_2:Dy (TLD200) thermoluminescent dosimeters

To improve our low dose capability we tested some chips of CaF_2 : Dy (3 x 3 x 0.9 mm). They have a linear γ-ray response from at least 0.5 mrad to greater than 1 rad and are highly reproducible. A standard deviation of better than 0.6% is possible with individually calibrated chips. Fading of the signal is about 25% per month; in reasonable agreement with other workers. Preliminary measurements have been made on their energy response to fluorescent X-rays.

Derived Working Limits for surface contamination by specific isotopes

We have now completed a thorough review of levels for skin and surface contamination for more than 30 isotopes of low toxicity. Of the isotopes considered only 3 are limited by inhalation of resuspended surface contamination. These are ^{63}Ni, ^{125}I and ^{210}Pb. We are at present

deciding what should be the appropriate skin thickness for the DWL derived from the dose to the epidermal layer of the skin. Originally 7 mg cm^{-2} was used but from work by Mrs Whitton of the CEGB, 4 mg cm^{-2} could be appropriate, particularly if the face is considered. The most probable choice will be the mean dose to a layer between 5-10 mg cm^{-2}. This data is being prepared for comparison with DWL's calculated previously. A final point is to decide the area which is contaminated. At the moment an infinite area is taken and this is acceptable for electrons, β rays, α particles but is unrealistic for X and γ rays. An area of 30 cm^2 could well be appropriate. The project is continuing.

Reference

Harvey, J.R., Hudd, W.H.R., Townsend, S.
Personal dosimeter for measuring the dose from thermal and intermediate energy neutrons and from gamma and beta radiation.
Neutron Monitoring for Radiation Protection Purposes. Vol II, p.199 (1973).

Publication

Comparison of general and specific derived working limits for surface contamination with reference to low toxicity isotopes.
J.A.B. Gibson, G.A.M. Webb, A.D. Wrixon.
Commission of the European Communities. Radiological Protection 4.
Radiation Protection Measurement Philosophy and Implementation, EUR5397.e.
p73 (1975).

Contractor:	Central Electricity Generating Board, Berkeley Nuclear Laboratories, Berkeley, Gloucestershire, England.
Contract No.:	078-74-7PSTUK
Head of Research Team:	Dr. B. M. Wheatley
General subject of contract:	Neutron survey equipment

Studies under this contract in 1974 identified a possible light-weight, wide range, rem-response monitoring system consisting of two components; one sensitive to neutrons with energies below 10 keV, the other to neutrons above 10 keV. In 1975 a component sensitive to high energy neutrons has been studied. It consists of a layer of scintillator of thickness 0.1 - 1 μm in contact with an hydrogenous plastic. A number of systems have been fabricated and tested with alpha and beta radiations and a computer program developed to predict the variation of sensitivity of such a device with neutron energy.

Results of project No.: 078-74-7PSTUK
Scientific staff: J. R. Harvey (0.3)
Title of Project: Feasibility assessment of a low weight, wide range, rem-response survey instrument

A possible two component neutron monitoring system identified in last years programme incorporates a low energy component consisting of a thermal neutron detector in a small moderator combined with a fast neutron detector utilizing a thin detector adjacent to an hydrogenous radiator. Since the low energy component presents no fundamental difficulty, attention has been focussed on a possible high energy component. The system initially chosen for study consists of a thin layer of scintillating material on a hydrogenous plastic base. Fundamental considerations suggest that the detector must have a thickness less than or equal to the range of 10 keV protons, which is the order of 0.5 - 1 μm in many scintillators.

In order to fabricate such layers a vacuum deposition system with equipment for monitoring the thickness of deposited layers was developed and layers of thickness between .03 and 0.3 μm fabricated from a number of inorganic scintillators including ZnS(Ag), CaF_2(Eu), CsI(Tℓ) and CsI(Ag). Of these, the most successful has proved to be CsI(Tℓ). The physical characteristics of layers of CsI(Tℓ) deposited at various rates have been studied using an electron microscope and the scintillation efficiency studied using a photomultiplier assembly and α and β radiation. The scintillation efficiency of the CsI(Tℓ) is approximately 2/3 that of the bulk crystalline material and the system should respond to neutron-generated protons of a few tens of keV with good discrimination against co-existent gamma radiation.

A computer program has been developed which will predict the pulse height distribution from such a device under neutron bombardment. The intention is to investigate various combinations of scintillating and hydrogenous layers to see which if any will give a rem-response for energies ranging between 10 keV and 10 MeV.

TRANSPORT VON RADIONUKLIDEN IN DEN KOMPONENTEN DER UMWELT

TRANSFER OF RADIOACTIVE NUCLIDES IN THE CONSTITUENTS OF THE ENVIRONMENT

CHEMINEMENT ET TRANSFERT DES RADIONUCLIDES DANS LES COMPOSANTS DU MILIEU AMBIANT

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Jahresberichten beschrieben:

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

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

100-BIAF CEA, CEN Fontenay-aux-Roses (Lafuma)

Biology Group Ispra

Contracting party of the Commission : Commissariat à l'Energie Atomique
BP N° 6, 92 260 Fontenay-aux-Roses,
France

Contract number :061-72-I PSAF

Chief od the Research Team : G. LACOURLY

General subject of the contract : LEVELS OF ENVIRONMENTAL POLLUTION

The purpose of the contract is to collect and incidentally, work out, the data and methods needed for evaluating in every point od the European Community, the acceptable limits of the radioactive pollution of the environment and the food chain, according to the parameters which determine the local characteristics.

In 1975, the five following projects have been carried on :

1/ Study of biological parameters of the European man
2/ Study of the parameters of environmental contamination from the atmosphere
3/ Study on the transfer to man of contamination resulting from water pollution and occurring during the transformation of raw products into human foodstuffs
4/ Study of the transfer parameters of the contamination, from soil and sediments to man
5/ Study on methods of assessing collectives doses with a view to application on optimaliging protection .

The project N°s 1, 2, 3 and 4 can be considered as virtually completed. The project n°.5 has to be considered as an orientation study for a future research.

PROJECT N° I

Project-Chief : L. KARLAUSEN

Project title : BIOLOGICAL PARAMETERS OF EUROPEAN MAN

Description of results -

I. Reference man :

The 480 pages report on standard man was published by Pergamon Press as ICRP publication number 23. It includes an anatomical, a physiological section and a third one which covers the elemental composition of the human body. Our group was mainly responsable for the anatomical section.

On the other hand, the contract with Professor Pribilla on the elemental composition of adult human body is finished and the results will be obtained soon.

2. Thyroid function - European Survey :

Twenty-four-hour-thyroid-uptake as well as urinary excretion of iodine were obtained on healthy euthyroid subjects in the six countries. Significant regional variations are observed. The data were collected from routine measurements done in different national health centres. The data will need to be standardized in order to allow proper comparison and interpretation. A manikin already used by IAEA for similar purposes will be used and circulated in the laboratories where the original measurements were made. This program is now concluded. The data are being analysed and we shall obtain them soon.

3. Effects of natural radioactivity on public health :

This is an epidemiological study purposing to evaluate the possible relationship between natural irradiation and the development of cancer. The purpose of the study is to study the dose-effect curve at low irradiation doses.

Preliminary results obtained in Brittany suggest that this region might be favorable for a pilot study. There are marked variations in natural radioactivity. Population is relatively stable. Mortality statistics are of very good quality and available. Finally, a first analysis of some data, performed by Dr L. Massé & Dr Pinces, showed a significant and unexpected correlation between mortality rates and natural radioactivity in some regions of the Côtes du Nord. During a first stage a general Survey of Brittany will be done to assess the distribution of natural radioactivity. This survey is part of a general Survey of natural radioactivity being conducted in France by the Atomic Energy Authorities. The analysis of mortality data will be done later.

This is a pilot study which will be extended in other parts of the EC countries.

PROJECT N° 2

Project Chief : L. ANGELETTI

Project title : STUDY ON THE PARAMETERS OF ENVIRONMENTAL CONTAMINATION FROM THE ATMOSPHERE

1. Retention of foliar applied iodine and strontium, on rye-grass and clover (collaboration with the Division of Biology, at Ispra)

The aim of the research was to evaluate the contamination of pastures, resulting from sprinkle irrigation of different intensity. The results that are being interpreted reveal the variability of the retention factor for the studied plants (rye-grass and clover) in relation with the sprinkling intensity. In the case of an one hour lasting drench, with an intensity increasing from 4 to 16 mm/h, by a constant concentrated solution, it was observed that the retention of strontium as well for the rye-grass, as for the clover increased with the drench intensity. On the other hand, the values of retention remain approximately constant for iodine.

2. Study on the transfer on grass of aerosols with various granulometry from atmosphere to soil (collaboration with the K.F.A., at Jülich)

The aim of these studies was to measure the deposition of aerosols on vegetation and conventional surfaces, according to the characteristics of aerosols, meteorological conditions and type of surfaces.

The studies were performed " in situ " with in the Jülich reactor (FRJ-1) activated copper sulphate, emitted 1,7 m above a parcel, partially cultivated with clover and partially with rye-grass. Many discshaped surfaces from 5 cm in diameter and made with filter paper and rough or polished metal were disposed above that parcel.

Starting from the obtained results, the deposition velocity was calculated following the equation ;

$$V_g = \frac{K}{I} \text{ (cm/s)}$$

K : contamination of the surface with the aerosol (Ci/m^2 soil surface)
I : integrated concentration of copper sulphate, 1 m above the soil ($Ci\ s/cm^3$ air)

Following the results obtained from 7 repeated tests, it appears that the deposition velocity on the artificial surfaces is generally lower than the velocity on rye-grass and clover. The same mean value V_g = 0,026 cm/s obtained as much for the soil as for the artificial surfaces seems to point out that the different roughness have no influence. The mean values of the deposition velocity, on the rye-grass V_g = 0,096 cm/s and on the clover 0,22 cm/s are higher than those on artificial surfaces, by a factor of 3,7 and 8,7 respectively.

Finally, the figure N°1 shows that the deposition velocity of aerosols on rye-grass depends from their diameter as well as from the friction velocity.

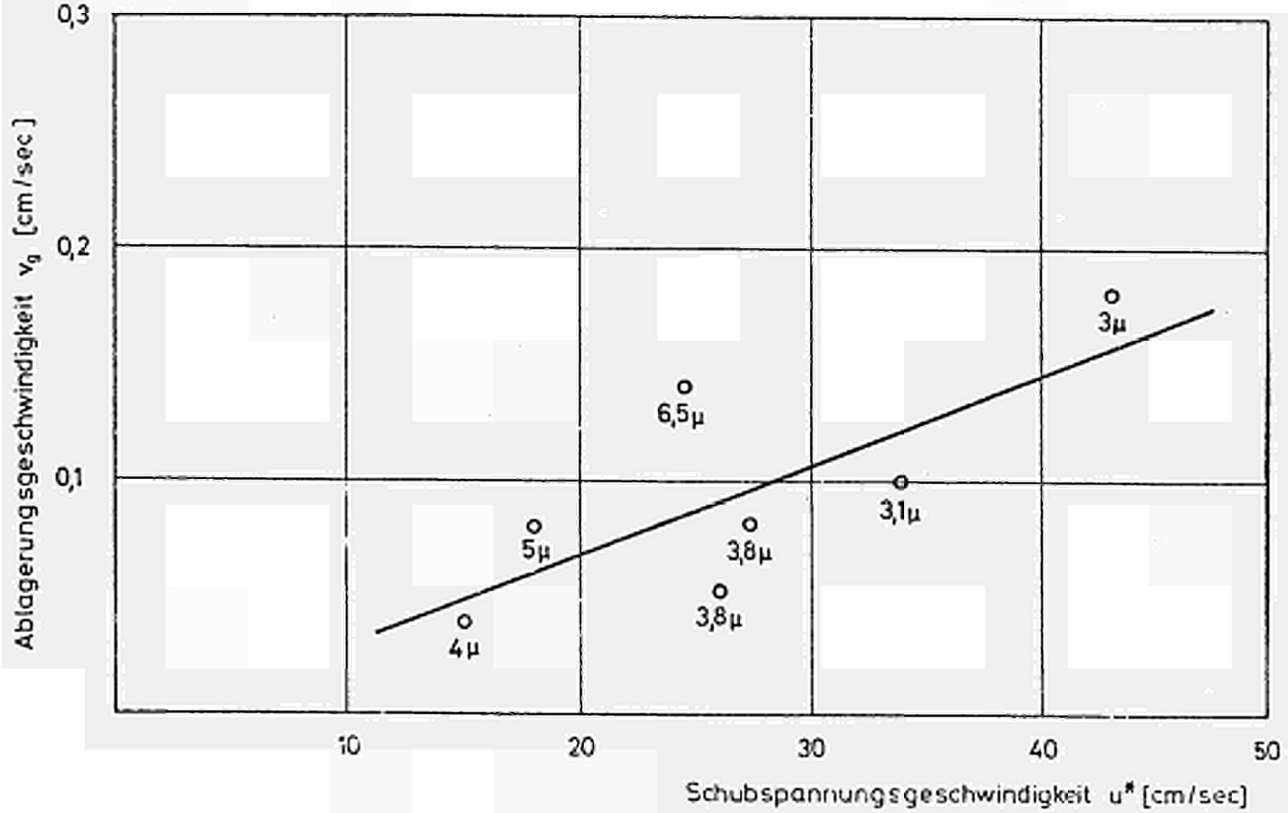

Abb. 1 Abhängigkeit der Ablagerungsgeschwindigkeit v_g von der Schubspannungsgeschwindigkeit u* auf Gras und bei verschiedener Aerosolgrößen

PROJECT N° 3

Project Chief and scientific fellow-workers : R. BITTEL, R. MAGNAVAL, A. GARNIER

Project title : STUDY OF THE PARAMETERS CONCERNING THE TRANSFER TO MAN OF CONTAMINATION RESULTING FROM WATER POLLUTION AND OCCURRING DURING THE TRANSFORMATION OF RAW PRODUCTS INTO HUMAN FOODSTUFFS

RESULTS

I. Study on contamination of irrigated ecosystems (collaboration with the Department of Biology - Euratom, at Ispra, and the Laboratory for Continental Ecology, DPr-CEA, at Cadarache)

Contamination of flooding irrigated rice-plantations :

Studies were devoted to a couple of very near elements : cadmium and zinc. The results obtained under controlled conditions (pilot model rice-plantations) ended in a common publication. These studies are now being completed by research on the linkage pattern of these two elements with the substrate in the rice grain. Concurrently, the contamination of Cyprinidae bred in such artificial rice plantations, was observed.

Contamination of plants irrigated by drench :

The study carried on at Cadarache about the incidence of stable cadmium and zinc on the contamination by 65 Zn of plants irrigated by drench were completed and results are being published.

2. Contamination of freshwater organisms (collaboration with the Department of Biology, Euratom, at Ispra and the Laboratory for Continental Ecology at Cadarache)

Transfer of stable cadmium and zinc 65 in freshwater benthic chain

A benthic chain of Lake Maggiore, from water to grubs and little fishes, was recreated in vitro. The results obtained are being interpreted.

Contamination of the eel by zinc 65 - Incidence of stable cadmium :

The laboratory of Continental Ecology had to cope with difficulties for the quantitative analysis of stable cadmium. After mastering these difficulties the adoption of an accurate analytical technique allowed to carry out the experimentation. Results are now being exploited.

Evaluation of the risk of mercury intoxication associated with tunny-fish consumption with the collaboration of the School of Public Health, Univeristy of California, Berkeley. This study was the subject of communication to the CENECA Colloquium and at the ESNA meeting at Cadarache (summer 1975). A publication written conjointly with the American staff is in preparation.

Relation between mercury and selenium in tunny-fish

The study carried on with the collaboration of the DPr-Cen-Far has been completed. The analyses of the results is in progress.

3. Study on the detriment for the environment

From a theoretical point of view, a bibliographical study led to propound the notion of critic subcellular organellae, on account of the microlocalisation of certain radionuclides (^{3}H, ^{14}C, Pu). Those results were presented at the International Conference in Molecular distribution and microdistribution of radioisotopes (Jülich, oct. 1975).

From an experimental point of view, studies on transfer-chains of heavy metals in marine environment have been extended by the study of microhistological consequences of pollution (study in progress at the INSERM-CERBOM, Institut at Nice).

The results of a study performed on the methyl mercury effect on rat liver deshydrogenases and oxydases have been published in " Experientia ".

4. Raw products pollutants transfer to foodstuffs on the level of human consumption (collaboration with the CEA laboratory for Plant Biology at Grenoble and the National Milling School at Paris)

The study of transfer of various metals (Mg, Fe, Zn, Cu, Cd, Sr, Pb, Co, Mo, Hg,) through cereal biological chains has been performed. A part of the results has been the subject of a communication to the ESNA Colloquium at Cadarache (summer 1975).

PROJECT N° 4

Project Chief and scientific fellow-workers : R. MAGNAVAL, R. BITTEL, G. LACOURLY

Project title : STUDY ON THE TRANSFER PARAMETERS OF CONTAMINATION FROM SEDIMENTS AND SOILS TO MAN

RESULTS

Study on the behaviour of Iodine in the soils (collaboration with the Division of Biology at Ispra)

This year, the research was exclusively devoted to the study of radioiodine, in order to elucidate the behaviour of Iodine-129 in the soil.

This behaviour was considered from three points of view :

1/ Iodine distribution in the system soil-solution
2/ Evaporation of iodine from soil surface
3/ Chemical form of iodine after evaporating from soil surface

1/ The distribution of iodine introduced in a system soil-solution, as $Na^{131}I$, has been measured for a group of seven European soil types (Eurosoils),for a lateritic soil and for clay (" illite ").

The isotherms of adsorption were linear in all the cases up to a concentration of 1×10^{-3} µg ml^{-1}, with a trend to a saturation curve for higher concentrations. The isotherm of the lateritic soil, with a high iron oxyde content (colcothar ?), was alone keeping his linearity up to a concentration of an iodine solution of 3.3 µg ml^{-1}. An acid pH accelerated the iodine adsorption by the soil. For radioprotection purposes, the concentration scale included in the linear part of isothermes only,has a practical interest. In that part, the maximum difference was seen between the behaviour of loess and the behaviour of " pseudo-gley ". For a given iodine solution concentration, the loess adsorbed four times more than the " pseudo-gley ", the other soils keeping an intermediary position besides the clay which adsorbed only a sixth of the loess.

2. Iodine evaporation from the soil surface has been measured during periods up to 11 days. Soil disks, one of loess and one of podzol have been exposed in a climatic chamber immediately after application of a $Na^{131}I$ solution. About 4% of applied iodine was evaporated in the first 5-6 days, whithout a supplementary loss up to the eleventh day.

It seems that the greatest part of iodine is already evaporated the first day after application. The concentration of the iodine solution (between carrier-free ^{131}I iodine and 100 $\mu g\ l^{-1}$) had no effect on the evaporation. On the contrary, the previous and entire iodine adsorption by the soil reduces his evaporation to a non-detectable level.

3. The chemical form of iodine evaporated from soil surface after application of a Na ^{129}I solution has been studied by gas chromatography. In the case of an by water oversaturated podzol, the presence in the atmosphere of $CH_3{}^{125}I$, $CH_3CH_2{}^{125}I$ and also $^{125}I\ O_3^-$ has been established, whereas molecular iodine could not be detected.

After moistening by a Na ^{125}I solution, up to the field-capacity, a podzol losed in gas form, only measurable quantities of $CH_3{}^{125}I$. The maximal concentration in the atmosphere was measured one hour after application. For a concentration of iodine solution of 10 $\mu g\ l^{-1}$, the evaporation velocity in the form of methyle iodide was in the first hour $5 \times 10^{-7}\ \mu g\ cm^2\ h^{-1}$. The evaporation from the surface of a loess was only a tenth of that of the podzol. In that case, no chemical form could be determined.

PROJECT N° 5

Project Chief and scientific fellow-workers : A. GARNIER, G. LACOURLY, P. PAGES, R. BITTEL

Project title : STUDY OF COLLECTIVE DOSES EVALUATION METHODS IN VIEW OF THEIR APPLICATION TO THE OPTIMALIZATION OF PROTECTION

RESULTS

The evaluation of detriment resulting from the irradiation of the population from a radioactive source involves, in the first place, an evaluation of collective doses. The studies carried on, to this aim in 1975, concern the 4 following points :

1. Study of evaluating methods of collective doses, on a regional scale, resulting from an atmospheric contamination.

The long distance atmospheric transport and the concentration of radioactive pollutants (research carried on with the collaboration of the Imperial College of Science and Technology at London - Dr A.J.H. Goddard and Dr H.H. Apsimon) has led to the working out of a prototype model. It consists to consider a release as a series of individual puffs, emitted at regular intervals (from one to six hours) and moving following a two-compartment model : the lower compartment where the mixing is effective ; the other, where the mixing is very weak, is considered as a series of 100 m thick layers. The calculation of trajectories is based on standard meteorological data recorded every 6 hours in Europe. It involves all the parameters on which the dispersion and the height variation of the mixing layer are depending and takes account from the various depletion processes. Testing this model in an example has given hopeful results, but revealed a lot of problems which have to be studied now.

Afterwards this model was applied to the evaluation of the collective dose on an European scale, by multiplying by each other the individual doses and the number of persons in every mesh of a grid covering the whole territory.

2. Evaluation of collective doses from an aquatic environmental pollution

This study was started with a bibliographical research on dispersion models and ecological parameters.

3. Study on the dilution factor of radioactive contamination of food products resulting from processing and exchanges

The general methodology applied to the case of milk products, following the French statistical data, allowed to evaluate the magnitude of any factors at the level of milk-collecting and processing. At the level of exchanges, the situation is very complicated, but the evaluation of manufactured products can be simplified. The adequate data are being collected in view of a bibliographical work gathering quantities of milk products and milk production, processing

and exchanges for any consecutives years in the Community of Nine.

Cereal products have been studied following the same principle starting from statistical data concerning the production and the exchanges of soft wheat and flour. The dilution factors intervening in the course of flour milling and breadmaking processes have been deduced from experimental studies on the transfer of heavy metals.

4. Study of a general methodology for optimalizing the public protection

The application of the CIPR recommandations concerning the maintenance of doses at a level " as low as reasonably achievable " leads to search for an optimalization of protection conditioned by the idea of an " acceptable burden for the society, based on the notion of detriment ".

The evaluation of the detriment involves the determination of the collective dose.

In order to explain various aspects of the general methodology, we have evaluated, as an example, for a French average area, the annual collective doses resulting from continuous releases of noble gases or 131 iodine, and have suggested particularly a realistic methode for evaluating the dose to the thyroid resulting from fresh milk consumption by the population.

PUBLICATIONS

EUR 5065 f (I974) Etudes relatives aux niveaux de contamination radioactives de la chaîne alimentaire et du milieu ambiant (I96I-I97I)

VOGT K.J., ANGELETTI L., GEISS H., HEINEMANN K. et al.
Untersuchungen zur atmosphärischen Ausbreitung und Ablagerung von Schadstoffen
Rapport Annuel juillet I973 - Juin I974 (KFA Jülich)

MAGNAVAL R., BATTI R., THIESSARD J.
Methylmercury effect on rat liver mitochondrial deshydrogenases
Experientia, 3I, 406-7 (I975)

LACOURLY G. La protection radiologique : Objectifs et analyse économique
Radioprotection, I975, vol. I0, N° 2, p I03-II9

BOVARD P., LACOURLY G., COULON R.
Prise en compte des problèmes thermiques dans les études écologiques et sanitaires préalables au choix des sites
IAEA, I975, SM-I87-32, p 76I-769

GARNIER A., BOUVILLE A.
Choix de la méthode d'évaluation de la contamination résultant des rejets atmosphériques d'une installation en fonctionnement normal, en fonction de l'étude des caractéristiques de l'environnement
IAEA, I975, SM-I80-I5, p 4I7-426

BATTI R., MAGNAVAL R., LANZOLA E.
Methylmercury in river sediments
Chemosphere (G.B.),I975, N° I, p I3-I4

VOGT K.J., GEISS H., HEINEMANN K., HORBERT M., MATTHES W., NORDSIECK H., POLSTER G.
Untersuchungen zur atmosphärischen Ausbreitung und Ablagerung von Schadstoffen. Ausbreitung von Schadstoffen in der Atmosphäre und Umweltbelastung
Rapport technique, 1er semestre I975, KFA Jülich

PALLY M., FOULQUIER L.
Introduction à l'étude de la fixation du cadmium par anguille anguilla (L)
Note CEA-N-I804, mai I975

ICRP N° 23 (co-auteur : KARHAUSEN)
Report of the task group on " reference man " (WS SNYDER, MJ COOK, ES MASSET, LR KARHAUSEN, G. PARRY HOWELLS, H TIFTON)
Pergamon Press, I975

HEINEMANN K., VOGT K.J.
Abschätzung einer mittleren Fallout Konstanten für elementares Jod au Gras aufgrund der Jülicher Ablagerungs-messungen - KST Bericht 2I4, Juillet I975.

HORBERT M., VOGT K.J.
Abschätzung einer mittleren Fallout Konstanten für Aerosole aufgrund der Jülicher Ablagerungs messungen
ZST Bericht 216, Septembre 1975

BITTEL R., MAGNAVAL R.
Microlocalisation des radionucléides artificiels et radioprotection du milieu
International Conference on molecular an microdistribution of radioisotopes and biological consequences
Jülich (RFA) 2-4 Octobre 1975

BITTEL R., FOURCY A., GARNIER A., LACOURLY G., MAGNAVAL R.
Utilisation d'une méthodologie mise au point pour l'étude des transferts des radionucléides au cas des éléments métalliques
Colloque International du CEMECA
Paris, 26-28 Février 1975

MAGNAVAL R., BATTI R., BITTEL R., BOUVILLE A., GUEZENGAR J.M.
Variation de la charge corporelle d'un toxique en fonction des habitudes diététiques - Evaluation par simulation numérique, du risque d'intoxication mercurielle associé à la consommation de poisson
Colloque International du CEMECA
Paris, 26-28 Février 1975

AUBERT M., BITTEL R.
Métaux lourds et chaînes biologiques marines,
3ème Congrès International " Décharges à la mer des eaux résiduaires urbaines et industrielles "
Sorrente, Italie, 23-27 Juin 1975

MAGNAVAL R., BATTI R., BITTEL R., BOUVILLE A., GUEZENGAR J.M.
Evaluation par simulation numérique du risque d'intoxication mercurielle associé à la consommation de thon
ESNA (European Society for nuclear methods in agriculture), Cadarache, 8-12 Septembre 1975

MAGNAVAL R., BATTI R., BITTEL R., BOUVILLE A., GUEZENGAR J.M.
Evaluation de la charge corporelle en fonction du régime alimentaire : Etude du risque d'intoxication mercurielle associé à l'ingestion de poissons
ESNA, Cadarache, 8-12 Septembre 1975

BITTEL R.
A propos de quelques problèmes récents posés par l'utilisation industrielle de l'énergie nucléaire
ESNA, Cadarache, 8-12 Septembre 1975

MOURIOUX G., FOURCY A., BITTEL R., GODON B.
Transfert de métaux lourds dans la chaîne de panification
ESNA, Cadarache, 8-12 Septembre 1975

AUBERT M., CAMAIN, PUEL D., BENIFMACHER J.P., BITTEL R., LAUMOND F.
Métaux lourds dans des chaînes biologiques marines naturelles ou artificiellement constituées - Plomb, mercure, cuivre, titane et vanadium)
ESNA, Cadarache, 8-12 Septembre 1975

BRESSON G., LACOURLY G.
La contamination alimentaire dans l'étude générale de l'environnement des sites nucléaires
Third European Congress of the International Radiation Protection Association (IRPA)
Amsterdam, I3-I6 Mai I975

BITTEL R. Incidence de pollutions chimiques sur les transferts des substances radioactives en milieu aquatique
Amsterdam, I3-I6 Mai I975

BITTEL R. Incidence de pollutions chimiques sur les transferts des substances radioactives en milieu aquatique
IRPA, Amsterdam, I3-I6 Mai I975

CARNIER A., Facteur de dilution de la contamination des produits alimentaires résultant des échanges et transformations entre production et consommation
IRPA, Amsterdam, I3-I6 Mai I975

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
IRPA, Amsterdam, I3-I6 Mai I975

MYTTENAERE C., MERLINI M., BITTEL R., DABIN P., MOUSNY J.M., POZZI G.
Etude de l'influence du cadmium stable sur le transfert du ^{65}Zn en écosystème irrigué par submersion (rizière irriguée)
AIEA, Colloque International sur les effets radiologiques des rejets des installations nucléaires dans les milieux aquatiques
OTANIEMI (Finlande), 30/6-4/7/I975

COMPOSITION DU COMITE DE GESTION

Président	Dr RECHT
Secrétaire	M. BRESSON
Membres	Dr JAMET
	M. DE SADELEER
	M. LAFFAYE
	M. VAN HOECK

COMPOSITION DU GROUPE DE RECHERCHES

Chef du Groupe	G. LACOURLY
Biologie humaine	L. KARHAUSEN
Etudes atmosphériques	L. ANGELETTI
Ecologie	R. BITTEL
	R. MAGNAVAL (jusqu'au 30 septembre 1975)
Etudes de synthèse	A. GARNIER
	P. PAGES
Documentation	R. HAMMER
	L. LAPORTE
Secrétariat	G. DEVENON

Contract No. 104-72-1 BIAI

Laboratorio Contaminazione Marina CNEN-EURATOM Association
Fiascherino (La Spezia) Italy

Dr. Aldo Brondi

Title:

The dynamics of radioactive and stable elements in the marine environment under special consideration of those elements which are important to marine radiocontamination

During 1975 the laboratory continued studies in the field of marine radiocontamination outlined in the program of the association contract.

The lines of research were directed towards the study of distribution of radiotracers in the different components of a marine ecosystem. Studies were carried on according to the different levels of the marine food chain; physical and chemical environmental factors, first trophic levels (phytoplankton), first heterotrophic levels (zooplankton and bacteria), last heterotrophic levels (crustaceans and fish). Unfortunately one project (zooplankton) is still suspended because the project leader has not yet been replaced.

In the same time all the groups of the laboratory were engaged in a survey, carried out with the Laboratory Vessel Odalisca, in the Archipelago "La Maddalena", where a nuclear base of the U.S. Navy is situated. The scope of this survey was to obtain environmental data in order to evaluate the receptivity of this specific marine environment.

Project No. 1

Title: Physical environmental factors of marine contamination and Special Developments

Name of Scientist: M. Bernhard (till the 15th of April 1976)

Results:

1) Development of an instrument computer system for the determination of number and size of fluorescent and not fluorescent particles

The redesigned apparatus has been tested. A report is prepared which describes the mechanical and electronical layout. First results on the counting of a unicellular alga Platymonas suecica are reported.

2) Simulation and model building

A technical report is practically finished on a program written in Basic which allows the simulation of compartment models not-in-steady-state up to 20 compartments. Simple examples of application are reported. Two persons of the group have taken part in a Fortran and T.S.O. course.

3) Instrumentation and apparatus needed by other groups

A program has been written for the Laben 70 computer for the evaluation of temperature and depths from reversing thermometers. A computer program has been written for the estimation of algal biomass taking into consideration the dimensions of various different species and their geometric configuration.

The laboratory has been linked to the CNEN computer center in Bologna via a terminal (Olivetti). With this terminal some programs have been run in order to check the operation of the terminal.

A program-timer has been developed and tested which automates in part the determination with the AMEL polarograph.

Project No. 2

Title: Investigation of the chemical factors influencing the distribution of the most important elements in the marine environment

Name of Scientist: A. Piro

Results:

1) Automatic determination of ammonia in seawater

A method for the continuous determination of ammonia in seawater has been developed using the Technicon Autoanalyzer. The method consists in the oxidization of ammonia to NO_3^-, followed by reduction to NO_2^- and determination via colorimetry. A solution of $K_2S_2O_8$ (0.5 %) was used for the oxidization of seawater samples in a thermostatic water bath at 80°C. The reduction from NO_3^- to NO_2^- has been realized by means of amalgam Cd-Hg column and of the colorimetric determination according to the method of Morris and Riley (1963). Since this method will be applied in future also for determinations in culture media and biological samples, studies are under way in order to overcome interferences of amino acids. A vertical profile of the distribution of NO_2^-, NO_3^- and NH_3^- in seawater (Ligurian Sea, 7 miles offcoast) is shown in Fig. 2.1.

2) Investigations of the interaction of zinc with marine sediments in relation to physico-chemical states

Experiences to evaluate the relationship between the grain size of sediments and zinc (in the form of Zn^{2+}) have been carried out. The results indicate proportionality between zinc adsorbed and surface.

3) Evaluation of chemical environmental factors in the La Maddalena Archipelago

During the cruise in the La Maddalena Archipelago the group was engaged in environmental surveys for the determination of those parameters which will be utilized for the environmental description and as support for other groups in order to get correlations (when and where possible) with other parameters (for example correlation between nutrients and primary producers etc.).

For the above purposes the following determinations were carried out:

a) determination of O_2;

b) determination of salinity;

c) discontinuous determination of nutrients ($N\text{-}NO_2^-$, $N\text{-}NO_3^-$, $P\text{-}PO_4^{---}$

d) continuous determination of NO_3^- and NO_2^-;

e) preparation of samples for the determination of heavy metals (Pb, Cu, Cd) and of other stable elements having possible corresponding important radioisotopes (Fe, Co, Mn, Zn) in organisms, sediments and seawater.

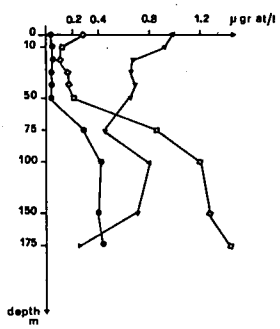

Fig. 2.1 - Vertical profile of $N\text{-}NO_2^-$ (●), $N\text{-}NO_3^-$ (◆) and $N\text{-}NH_3$ (▲) offshore the Gulf of La Spezia. The determination of $N\text{-}NH_3$ is till to now affected by an interaction due to aminoacids

Project No. 3

Title: The role of phytoplankton in the accumulation, loss and transfer of radionuclides

Name of Scientists: A. Zattera and L. Rampi (part time)

Results:

1) Uptake of zinc

Stable zinc in algae depends directly (see previous Annual Reports) from that contained in the media; for example when the stable zinc contained in the media increases from 5 to 100 µg/l the quantities taken up by algae at equilibria are always 50 % of those present in the media.

The question that can be posed is if all the zinc in the algae is really taken up by them or, if some portions of it, are present in the media in a particulated form, not directly associated with algae, which could be removed during the sampling procedure.

Experiments carried out in collaboration with the Chemistry Group show that a certain percentage of stable zinc added to the seawater media is particulated (total zinc added: 50 µg/l, particulated: 14 µg/l = 28 %). Electrolysis of zinc enriched seawater carried out at a pH 8.0 and with a potential of - 1.2 V shows that practically 100 % of zinc is electrolyzable; this indicates that both ionic and particulated zinc can be reduced and the particulated is adsorbed and not strongly linked. By adding Zn^{65} to natural seawater media, sterilized by UV exposure, we noted, 6 days after addition, that 40 % of the Zn^{65} added is in particulated form at the pH of the media ($8 \pm 0,1$). The quantities of particulated zinc decrease with decreasing pH till to pH 6 at which all the particulated is (probably) ionic.

This indicates that separation of algae from the media by centrifugation includes also a particulated fraction of zinc which is not due to algae. Various attempts, like centrifugation in density gradient with Ficoll and Rompacon, to separate algae from the particles have been made without success.

2) Diffusion processes in La Maddalena Archipelago

During 1975 the group was interested in evaluating the diffusive properties of the La Maddalena Archipelago (Sardegna Island) where a nuclear base of the U.S. Navy is situated. The studies were conducted with istantaneous releases of Rhodamine B as tracers for water masses. The results show that the behaviour of the maximum of the concentrations with time are fitted by an equation of the type:

$$C_{(max)}(t) = Kt^{-2}$$

while the spatial behaviour of the concentration seems to fit well the Okubo-Pritchard model the equation of which is:

$$C(r,t) = \frac{M}{\pi \omega^2 t^2} \quad e^{-\frac{r}{\omega^2 t^2}}$$

where $C(r,t)$ is the concentration at time t and at a distance r from the maximal concentration. t is the time and is the "diffusion velocity". The last equation is a form of the following:

$$C(r,t) = C(0,t) \exp \left[-b(t) r^m\right] .$$

The results of the spatial distribution summarized in Fig. 3.1 indicate, as already said, that the Okubo-Pritchard model is fitted.

3) Distribution of phytoplankton and phytobenthos in the La Maddalena Archipelago

The distribution of phytoplankton in the Archipelago was estimated over 13 stations and was found highly heterogeneous as can be seen from Tab. 3.1, in which the concentrations of the major groups of phytoplankton in the water column are reported. At present no correlations between phytoplankton concentrations and most important physico and/or chemical parameters can be supplied.

Benthic algae are represented by a few species only: Halimeda tuna with a biomass ranging from 40 to 2400 g/m^2, Caulerpa prolifera from 100 to 500 g/m^2, Acetabularia mediterranea and Padina pavonia in negligible amount. On the contrary the plant Posidonia sp is present with a biomass from 3000 to 20000 g/m^2.

Table 3.1 Distribution (cells/l) of the major groups of phytoplankton on 13 stations of the La Maddalena Archipelago

	0 - 15 m depth		15 - 30 m depth	
Not derminable cells	260 000-	2 200 000	400 000-	1 300 000
Diatomeae	850-	20 000	760-	3 800
Peridineae	1 800-	7 500	1 700-	6 400
Coccolithophorideae	200-	2 800	100-	1 300

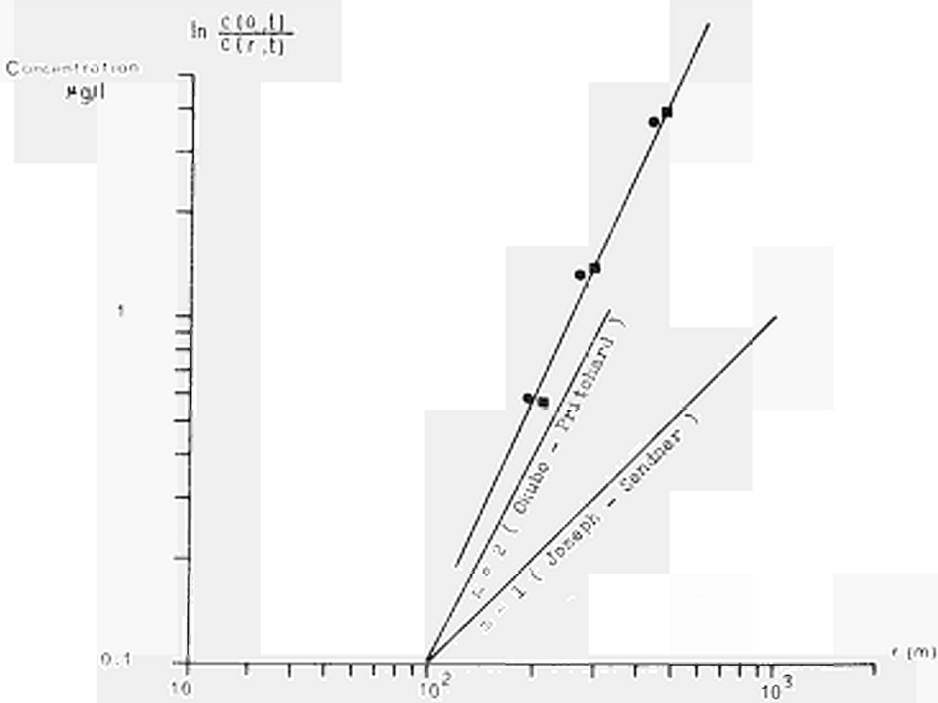

Fig. 3.1 - La Maddalena Archipelago (S. Stefano bay). Diffusion behaviour comparing the slope of the data obtained from two (o x) istantaneous releases with the Joseph-Sendner and Okubo-Pritchard models 8 h after the release.

Project No. 5

Title: The role of the last levels of the food chain (mussels, crustaceans, fish) in the accumulation and transfer of radionuclides relevant to marine radiocontamination

Name of Scientist: E.H. Schulte

Results:

1) Influence of temperature on the survival of prawns

In first experiments the influence of temperature on the survival of prawns has been studied in order to estimate the thermal impact in the marine environment.

Leander squilla juveniles (total length 2.5-3.5 cm) have been acclimatized to different temperatures (25, 30, 35°C) by raising the temperature of 1°C daily. Then the specimens were transfered to aquaria with lower or higher water temperatures than the acclimatization temperature.

A change of temperature from 25 to 10°C and also to 5°C caused no mortality over 24 hours nor over 115 hours of exposure, while all specimens died within 5 minutes when transfered from 25 to 40°C. Same results were obtained with an acclimatization temperature of 30° and 35°C. No mortality has been observed when the temperature was changed from 30 to 20 and 10°C and from 35 to 15 and 10°C. In all these experiments the survival rate amounted to 100 % still after 48 hours and in some cases also after 96 hours.

On the other hand 100 % mortality has been observed in experiments where the temperature exceeded 35°C. A change of the acclimatization temperature of 35 to 40°C resulted in a mortality of 100 % within 5 to 15 minutes after the start of the experiment. Dying specimens changed first colour from transparency to opaque and white starting with the outer-most part of the abdomen and then get immobile.

In a further experiment specimens of Leander squilla, acclimatized to 25°C, were transfered to aquaria with a water temperature of 35°C which caused also a mortality of 100 % within 24 hours. These experiments showed that 35°C represented a critical temperature for the survival of prawns, since when this temperature was exceeded or reached by transfer of specimens from a lower temperature to 35°C, a mortality of 100 % had been observed.

The long-term effect of temperature on the survival of prawns was studied acclimatizing juvenile Leander squilla to 35°C by raising the temperature of 1°C daily. After reaching 35°C the temperature was maintained constant for more than two weeks, and dead specimens were counted daily. The results of the experiments are summarized in Fig. 5.1. As can be seen the mortality rate of 50 % was reached after 10 days of exposure to 35°C.

2) Distribution of zoobenthos and nekton in the Maddalena Archipelago

In 12 litoral stations the distribution and frequency of zoobenthos have been studied considering fixed areas of 0,5 m^2 and o,25 m^2. The most representative classes have been found to be Polychaeta, Crustacea, Echinoidea, Bivalvia, Gastropoda while the others (Bryozoa, Holothurioidea, Ophiuoridea, Ascidiacea, Anthozoa, Sipunculida, Placophora, Porifera, Asteroidea) were distributed in small numbers only, and hence contributed only to a very little extent to the biomass of the samples. The samples of all stations contained too little numbers of zoobenthos for a valid calculation of the biomass/m^2.

In the Maddalena zone fishing is performed mainly with gill-nets using small fishing boats of 56-20 HP (9 boats) and of 19-4 HP (23 boats). For these two groups of fishing boats the catches per day per boat have been calculated considering calm days only (wind velocity between 0-5 km/h) which were 42 days in 6 months.

The amount of fish caught by the bigger fishing boats was calculated to 31.6 kg/day/boat while for the smaller ones 18 kg/day/boat were found. The total catches landed at the Maddalena port amounted to 53180 kg in 6 months, considering also two other types of fishing boats, which caught 108.4 kg/day/boat and a small trawler with a daily catch of 307 kg.

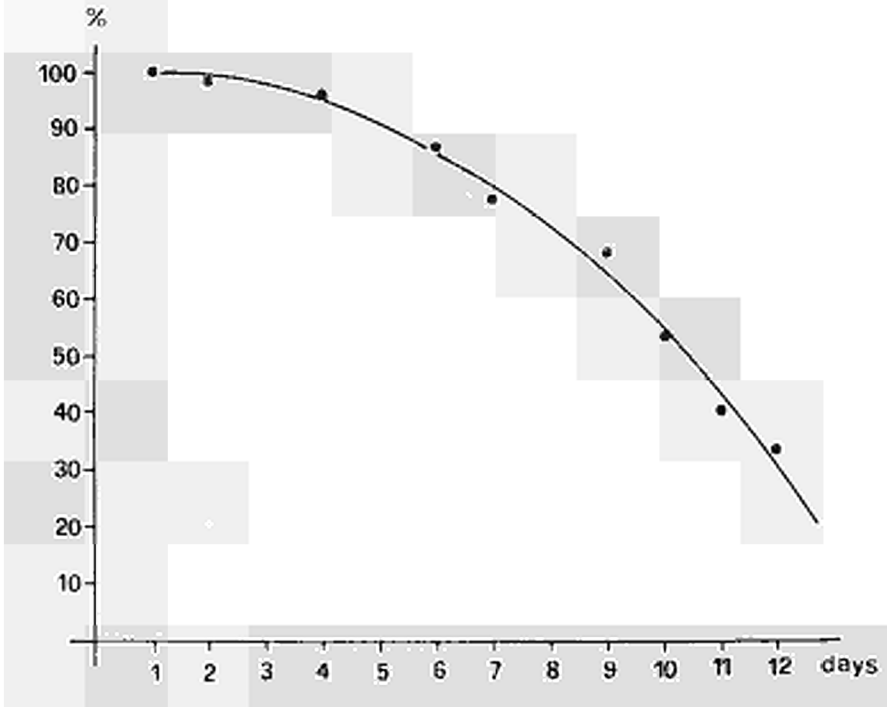

Fig. 5.1 - Survival (%) of Leander squilla juveniles acclimatized to 35°C water temperature within 15 days by raising the temperature of 1°C daily.

Project No. 6

Title: The role of heterotrophic level of microorganisms in the uptake and transfer of a few ecologically relevant radionuclides and distribution of metabolically active bacteria in the marine environment

Name of Scientist: C.N. Peroni

Results:

1) Origin of autoradiographic spots from natural marine populations of microorganisms

Experiments concerning the origin of autoradiographic spots from natural marine populations of microorganisms were carried on by comparing the spots obtained by bacteria and algae.

The bacterial strain λ and the flagellate β_2 were incubated in UV-irradiated radioactive (^{32}P) seawater for 12 h. From Fig. 6.1, it can be seen that the flagellate β_2 gives great, clear and uniform spots (L), whereas the bacterial strain λ produced spots (A) of smaller and various sizes. Blank, i.e., UV-irradiated radioactive seawater, gives the autoradiographs shown in Z.

Therefore, the sharp difference visible between the two types of spots is not enough to give objective criteria for distinguishing the bacterial and the algal spots in natural samples. However, in counting autoradiographs deriving from the filtration of 0.2 ml of natural samples (see previous Annual Reports), the number of spots having a diameter greater than the diameter of spots given by the control autoradiographs made with the bacterial strain λ is very low (< 2 %) compared with the spots of smaller sizes which, according to us (Peroni and Lavarello, 1975), are given by bacteria present in the sample.

In passing, it was also noted that algal cells (β_2) inactivated by UV for 5 min do not take up any ^{32}P even after an incuba-

tion time of 18 h.

2) Bacterial zinc content

The zinc content of bacterial cells was determined by polarographic analysis of a dense bacterial culture after centrifugation. A mean content of $\sim 5.10^{-12}$ µg Zn/one cell was obtained for the tested strain (λ).

3) Participation to the Maddalena program

A member of the group participated to the "Maddalena survey" collecting samples for direct microscopic counts.

The better technique of analysis is now under study. Tests have been made with the erythrosin staining technique (Sorokin and Overbeck, 1972) and the fluorescence staining by acridine orange of filters using Nuclepore membrane filters (Zimmermann, 1974).

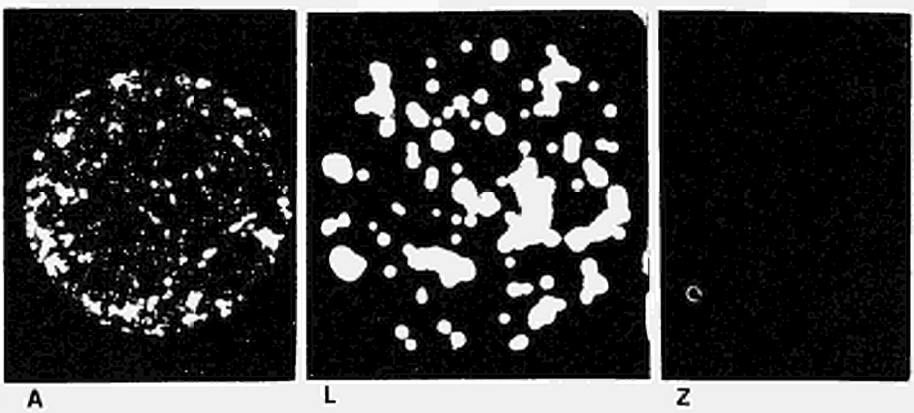

Fig. 6.1 - (See explanation in text).

Management Committee

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Collaborations and participations in Scientific Meetings

As in the past the Laboratory has collaborated with the Istituto di Zoologia (Prof. Schreiber) Parma, the International Laboratory for Marine Radioactivity (IAEA) Principality of Monaco and the Center of Marine Research (Institute Ruder Boskovic) Rovinj, Yugoslavia.

Members of the Laboratory took part to the following meetings: Expert Consultations "Pollution in the Mediterranean" FAO-USECO-WHO-UNEP (July-September); VII Congresso Nazionale della Societá Italiana di Biologia Marina (21-24 May, Venice); 10th European Symposium on Marine Biology (17-23 September, Ostend); Symposium on the Interaction between Water and Living Matter (10-13 October, Odessa); Congresso Annuale delle F.E.P.A. organized by the FAST "Risorse Biologiche delle Acque" (22-24 October, Venice); Symposium on Radiological Impacts of Releases from Nuclear Facilities into Aquatic Environments (Helsenki).

Publications prepared during the year 1975

BERNHARD, M., 1975 - Studies on the radioactive contamination of the sea. Annual Report 1973-74. (In Press).

BERNHARD, M., and A. PIRO, 1975 - Zinc in seawater: An overview 1975. In: "The Nature of Seawater". E.D. Goldberg (Ed.). Physical and Chemical Science Report 1, Dahlem Workshop Report, Berlin, pp. 43-68.

BERNHARD, M., and A. ZATTERA, 1975 - Major Pollutants in the Marine Environment. In: Marine Pollution and Marine Waste Disposal. Pearson and Frangipane (Ed.) Pergamon Press - Oxford and New York, pp. 195-298.

BERNHARD, M., and A. ZATTERA, 1975 - Valutazione per alcuni polluenti delle concentrazioni tollerabili nell'ambiente marino e l'ingestione limite per l'uomo. In: Ecologia acque, aria, suolo - Vol. 8 pp. 612-620.

BERNHARD, M., and A. ZATTERA, 1975 - La distribuzione di alcuni polluenti nell'ambiente marino con tentativi di stima delle conseguenze. Presented at the 7th Congress od the Italian Society of Marine Biology. (In Press).

BERNHARD, M., and A. ZATTERA, 1975 - The role of chemical speciation in the uptake and loss of elements by marine organisms. Presented at the Symposium "Interaction between water and living matter" Odessa USSR. (In Press).

SCHULTE, E.H., 1975 - The laboratory culture of the palaemonid prawn Leander squilla (L.). In: Proceedings of the 10th European Symposium on Marine Biology. (In Press).

Contractant van de Commissie: Institute of the Association EURATOM-ITAL, Wageningen, the Netherlands.

Nummer van het contract: 094-72-1 BIAN

Hoofd van de groepen voor onderzoek: Dr.Ir. D. de Zeeuw.

Algemeen onderwerp van het contract:

RADIATION PROTECTION

- Movement of radioactive pollutants in soils.
- Uptake of radioactive pollutants by plants.
- Radiation effects (physical, genetical, biochemical).

Algemene omschrijving van de uitgevoerde werkzaamheden:

Main topics of the 1975-research by the soils and plant groups of the Institute were:

- continuation of sampling and analysis for the experimental control of the mathematical model concerning the behaviour of ^{90}Sr and ^{137}Cs in soils of Western Europe.
- study of the behaviour of metals (potential radiocontaminants (e.g. ZN, Mn, Cr, Co), as well as related pollutants (e.g. Cd, Cu, etc.) in soils and sediments of selected sites where these elements are present in higher concentrations and hazards of pollution of groundwater, surface water and vegetation therefore exist.
- root and foliar uptake of Cd (interaction with ^{65}Zn), its binding to cystein related to the uptake, its pattern of lateral transport in plant stems, the effect of the interaction with Mn and Zn on leaf accumulation and of Cd itself on steps in the photosynthetic reaction in isolated chloroplasts. A theoretical model for explaining the multiphasic shape of the absorption isotherms observed in studies of the kinetics of root uptake of metal ions, was worked out.

Topics in research on radiation effects in plants and related material were:

- radiation-induced chromosome rearrangements in *Haplopappus gracilis* (Nutt) Gray.
- irradiation effects on the survival of pollen of different species.
- analysis of radiogenetic effects in the spidermite (*Tetranychus urticae* Koch.) on the basis of the molecular theory, which has been further controlled and extended, using data from the first topics and from literature.
- further development, improvement and application of dosimetric techniques, mainly considering the lyoluminescent properties of various saccharides and conditions for the use of thermoluminescent materials and perspex dosimeters.

The programme for 1975 has once more been carried out in close cooperation with other scientific institutes and organizations. Examples of this collaboration are:

- on different aspects of the application programme within workinggroups of the European Society of Nuclear methods in Agriculture (ESNA);
- on pollution, radioactive and other, with the Biology group at Ispra and institutes in the Netherlands, Belgium and Germany;

- on radiation effects within the European working group for Microdosimetry;
- on standardization of absorbed dose and dose distribution measurements within the European Late Effects Project Group (EULEP).

INSTITUTE OF THE ASSOCIATION EURATOM-ITAL
P.B. 48, Wageningen, The Netherlands.

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Changes in the Scientific Staff

Ir. P.W.F. de Vrijer left the Institute to accept research duties elsewhere.
Temporary members (post-graduate fellows) responsible for particular aspects of the programme: ir. M.A. Daelemans, dr. A.W. Spanjers, G. Zurlini, M.Sc., dr. E.M. Perea Dallos, dr. T. Luczkiewicz.
Several guest-workers have spent 6 to 12 months at the Institute.

Resultaten van het project No. 1

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra.

Titel van het project:

Verification of predictions concerning Sr and Cs behaviour in soils in Western Europe.

Beschrijving van de resultaten:

At its final stage, this project mainly aims at collecting, over the years, enough analytical data from annual samplings for comparison with the predictions of the simulation model. Analytical work concerning ^{137}Cs and ^{90}Sr are ready now for 90%; the final data will be available in the course of 1976. A first verification of the usefulness of the model can then be started.

Resultaten van het project No. 3

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra, D. Mulder, N.v.d.Klugt.

Titel van het project:

Behaviour of heavy metals in soils.

Beschrijving van de resultaten:

The research in this project was again focused on heavy metals among radioactive contaminants and their associated stable isotopes as well as on related elements in this group (e.g. ^{65}Zn, Zn, Cd). As methods, mainly neutron activation analysis and atomic absorption spectrometry, now available, permit, almost without additional costs, the determination of a whole group of other pollutants, they were included in the programme and therefore in the report. Another characteristic of this report is that the problem as stated in the title was focused in 1975 on selected areas and situations where contamination may in fact occur. To the latter belong: Subsoils of a refuse dump at Delden (Netherlands); Soils used to dispose sewage water at Braunschweig (FRG); Soils of the delta of the river Rhine (Biesbosch, Valburg, (Netherlands)); Soils of the Veluwe which are destined to be used as a drinking water reservoir (for the infiltration use will be made of purified water of the river Rhine (Netherlands)); Deposits of lake Nakura (Kenia); Reference soils. As most of the processes, responsible for binding of heavy metals in soils, are sensitive to changes in the redox potential, part of the investigations were devoted to oxygen measurements.

Contamination of the subsoils of the refuse dump Delden (Netherlands).

The project is carried out in cooperation with the "Stichting Verwijdering Afvalstoffen"(SVA) at Amersfoort. At the refuse dump the system of controlled tipping or sanitary landfilling is used. Two samples were taken from below the dump, respectively to depths of 280 and 140 cm; a reference soil was sampled to 200 cm depth.
From this reference soil, only the analytical results for the upper 100 cm are now available; this limites the conclusions present.
It is quite clear that the upper 100 cm of the soils below the refuse dump are not contaminated (see table 1). From a depth of 70 - 100 cm on a small increase of the levels of Co and Cr can be observed. This may indicate that migration through the upperzone is possible, while therebelow precipitation occurs. Such a difference in behaviour may be explained by (a) decarbonized upper layers, carbonate containing lower layers, (b) anaerobic upper layers, aerobic lower layers.
Decarboxylations may have occurred through CO_2 production originating from the former vegetation or from the effluent of the dump. Anyway pH and $CaCO_3$ content are so much lower in the upper layer that they may explain a reduction in adsorption capacity (less possibilities for Ca precipitation on iron-aluminium-manganese hydrous oxides, less possibilities for carbonate precipitates). However, low redox potentials reduce the amount of hydrous oxides. Definite conclusions can only be made after analysis of the reference samples. For the time being explanation (a) is favoured.

Table 1 - Soil characteristics of subsoils of the refuse dump at Delden. Heavy metals determined by nuclear activation analyses. Other characteristics determined by A. Breeuwsma (Stiboka). Typical data for refuse dump site 1, all values in ppm unless indicated otherwise.

Depth (cm)	Al	Ce	Co	Cr	Cs	Eu	Fe	Hf	Hg	Mn	Sc	Tl	V	Zn	Cd	pH (kCl)	$CaCO_3$ %	CEC	Fe^{++},wet	Fe^{+++},wet
																		me per 100 g		
0 - 5	28440	34	2,1	41	1,3	0,4	10930	8,3	0,12	240	0,7	1325	22	82	0,26					
5 - 15	19920	29	1,8	37	1,1	0,3	9150	7,8	0,11	210	0,6	1055	12	60	0,27					
15 - 25	16340	22	0,9	22	0,7	0,2	5160	7,3	0,03	140	0,4	890	6	29	0,12					
25 - 35	18560	17	0,7	22	0,7	0,2	4240	7,7	0,03	120	0,4	735	< 1	32	0,09					
35 - 45	20370	16	0,9	18	0,9	0,2	5340	5,9		150	0,4	685	< 1	26		4,71	-	8,46		
45 - 55	22860	15	0,8	20	0,8	0,2	2880	3,8		90	0,3	635	< 1	20		4,75	-	7,72		
55 - 65	22770	17	0,9	21	0,8	0,2	3030	5,8		80	0,4	685	< 1	29		4,76	-	4,86		
65 - 75	21540	19	1,1	17	0,8	0,2	2900	4,1		70	0,3	720	< 1	27		5,21	-	2,21	2,81	
75 - 85		6	1,1		0,5	0,3	2130	6,5			0,4			13		5,28	-	2,76	3,57	
85 - 95		6	1,3		0,7	0,3	2680	8,3			0,6			-		5,75	-	2,65	3,46	
95 - 110		23	12,3		2,2	1,2	7460	5,1			1,6			-		5,54	-	2,93	5,59	18,2
110 - 120		18	6,0		1,8	1,0	8490	3,6			1,4			-		7,40	13,4	1,14	20,1	53,4
120 - 140		16	11,9		2,4	0,8	10130	2,2			1,6			-						
140 - 160		12	6,6		2,3	0,8	8340	2,4	0,00		1,5			46						
160 - 170		17	5,5		2,0	0,8	7980	2,9			1,6			-						
170 - 200		8	4,5		1,3	0,6	4730	1,4			0,8			24		7,70	8,0	0,23	5,50	23,4
200 - 230		-	5,2		1,4	0,6	4940	1,5			0,8			23		7,75	7,3	0,20	6,73	24,6
230 - 260		-	5,4		1,6	0,6	5490	1,5			0,9			27						
260 - 270		8	3,4		1,8	0,4	3040	0,8			0,5			18						
270 - 280		9	4,4		1,6	0,5	4070	1,2	0,00		0,7			20						

Contamination sediments lake Nakura (Kenya).

In cooperation with Prof.Dr. Koeman we investigated the Cu and Zn content of a few sediment samples from lake Nakura, Kenya. The water in this lake is supplied by a river, but the lake has no outlet. The sewage treatment installation of the city of Nakura releases its water into this lake too. The pH of the lake-water is 10.5. *Spirulina plentensis* (Nordstedt) Geitler is the only type of algae present in the lake. These algae are food for flamingoes, and, indirectly, also for pelicans. In both birds the Cu and Zn content is relatively high. The sediments were analysed to check whether the sewage water may be the reason for the high Cu and Zn contents. (see table 2).

Table 2 - Heavy metal contents (in ppm) of sediments of Lake Nakura.

Sample		Cu*	Zn*	Zn	Cr	Co	Fe	Sb
1	Sewage out	5,6	230	280	11	2,6	57380	1,1
2	Sewage out	9,0	280	250	16	2,3	52380	1,3
3	Lake, close to the outlet	7,0	280	210	14	2,3	50500	-
4	Lake, close to the outlet	7,3	280	210	15	2,2	47600	0,9
5	Lake, close to the outlet	5,6	270	200	14	2,3	49700	-
6	Lake, close to the outlet	5,2	250	180	11	2,0	44900	-
7	Lake, far from the outlet	4,3	250	170	12	2,1	42700	-
8	Lake, far from the outlet	4,7	260	190	13	23,	47700	-
9	Lake, far from the outlet	4,9	260	190	14	2,3	47800	-
10	Lake, far from the outlet	2,3	190	170	13	2,4	48200	-
11	Lake, far from the outlet	2,9	230	180	15	2,5	48100	-
12	Lake, far from the outlet	3,3	190	120	13	2,2	37600	-
13	Lake, far from the outlet	3,4	170	120	12	2,1	36600	-

*Atomic Absorption, other values Nuclear Activation Analysis.

Comparing these figures with the Cu and Zn contents of other soils (Cu: Alkmaar 15 ppm, Valburg 90 ppm, Biesbos 300 ppm; Zn: Alkmaar 70 ppm, Valburg 850 ppm, Biesbos 2500 ppm) both the Cu and Zn level are rather low. Dr. Duinker, who investigated the Cu and Zn levels in the lake-water, also found low Zn and Cu levels. Further investigations, in which especially the high pH of the lake and the chemical form of the elements have to be considered, seem necessary to explain the relatively high Cu and Zn contents in the birds.

Contamination of soils of the delta of the Rhine river.

Survey of heavy metals in different soils of the delta of the Rhine was continued. Cu, Mn, Cd and Ni were determined.
The soil from Alkmaar is a reference soil used for normal agricultural practice (permanent pasture), situated in the North of the province of Holland. The figure for Cd contains revised values for the Biesbos.
Soils of the Biesbos area contain the highest amount of contaminants: levels in soils from the forelands (Valburg) are much lower.

Sewage water disposal fields at Braunschweig (FRG).

Investigations were concentrated on (a) the determination of possible accumulation of heavy metals, and (b) on migration studies.

Table 3 shows that accumulation of Zn, Cd, Ag and Sb occurred in the top soil. At lower levels there is no accumulation at all; one gets even the impression that the levels are lower than in the reference soil (table 4).

Table 3 - Distribution heavy metals (ppm) sewage water disposal field at Braunschweig.

Depth, cm	Ce	Co	Cs	Eu	Fe	Hf	Mn	Sc	Zn	Ag	Sb	Cd
0 - 10	10	3,5	1,3	0,4	6500	6,4	250	3,1	340	3,5	2,3	}2,76
10 - 20	-	3,4	1,3	0,4	6600	6,5	280	3,3	340	3,3	2,0	
20 - 30	-	3,6	1,3	0,4	7100	5,8	320	3,4	316	3,4	2,0	3,29
30 - 40	-	3,3	1,3	0,5	6600	7,0	285	3,4	164	2,0	-	0,98
40 - 60	16	4,2	1,4	0,5	6800	7,0	275	3,6	67	0,7	0,9	0,42
60 - 80	12	4,4	1,5	0,5	7600	5,3	400	3,8	48	0,5	-	
80 - 100	-	3,7	1,4	0,5	6800	4,6	315	3,4	39	-	-	
100 - 120	-	3,3	1,0	0,4	5400	3,6	370	2,4	30	0,3	-	
120 - 140	-	2,3	0,9	0,4	4300	4,3	265	2,0	21	-	-	
140 - 160	-	2,3	0,8	0,3	4900	4,5	220	-	25	-	-	
160 - 225	8	1,6	0,7	0,3	4300	2,0	85	1,4	23	-	-	

Table 4 - Distribution heavy metals (ppm) reference soil at Braunschweig.

Depth, cm	Ce	Co	Cs	Eu	Fe	Hf	Mn	Sc	Zn	Ag	Sb	Cd
0 - 10	11	3,8	1,0	0,4	10100	4,2	510	2,1	60	-	0,6	0,36
10 - 20	11	4,0	1,0	0,4	9800	4,8	525	2,2	78	0,4	0,7	0,40
20 - 30	11	3,7	1,0	0,4	9900	4,6	500	2,1	78	0,5	0,8	0,27
30 - 40	14	4,7	1,2	0,5	9700	5,3	485	2,6	84	-	0,6	0,10
40 - 50	17	6,1	1,4	0,6	12800	6,1	520	3,5	92	-	-	0,08
50 - 60	18	6,4	1,7	0,6	15500	5,4	440	4,2	104	-	-	0,06
60 - 70	14	4,8	1,4	0,5	12400	4,2	330	3,5	74	-	-	
70 - 80	13	4,4	1,5	0,5	13970	3,5	190	3,6	80	-	0,4	
80 - 90	10	3,9	1,3	0,4	11920	1,9	200	3,0	63	-	-	

Comparing these data with those of the refuse dump we apparently get a paradoxal situation:
- Refuse dump, low effluents, contamination at greater depth,
- Sewage field, large effluents, contamination in topsoil.

Analysis of the reference soil at greater depth will have to be carried out to clear this point. Otherwise it may well be that, at Braunschweig, a situation is developed in which almost no further accumulation of Zn takes place, but in which Zn is leached to lower subsoils (or to the canal north of the sewage fields). From the soil solumn studies, performed with ^{115m}Cd and ^{65}Zn it has become clear that both Cd and Zn do migrate through the soil (see figs. 2 and 3).
Furthermore it appeared that Cd and Zn are almost for 100% reversibly adsorbed. A further mathematical analysis of the data will be carried out next year. Fig. 4 shows some adsorption curves for Zn. Also from this figure and the figure for Cd, reported in the 1974 annual report, it appears that both Zn and Cd are not adsorbed, to a considerable extent, by the Braunschweig soils.

Possible contamination of the subsoils of the Veluwe upon infiltration with purified Rhine water.

The Association's Institute was asked by the Netherlands Government Institute for Water Supply (R.I.D.) whether it was possible to predict the behaviour of heavy metals in Veluwe subsoils upon infiltration with purified Rhine water. The level of heavy metals in the purified water will be low; expected levels are Cd 0,5 µg/l, Cr 2 µg/l, Cu 10 µg/l, Hg 0,2 µg/l, Pb 5 µg/l and Zn 40 µg/l. These levels are apparently very acceptable. Undesired situations may occur when, in some way, first adsorption and afterwards, all of a sudden, desorption takes place. Such a situation is to be expected if the subsoil passes from aerobic to anaerobic conditions. Under aerobic conditions coprecipitation of part of the heavy metals on iron-aluminium-mangenese hydrous oxides will occur, under anaerobic conditions such hydrous oxides will go into solution. The turnover from aerobic to anaerobic conditions is controlled by the oxygen concentration and redox potential of the soil. At R.I.D. almost no information was available on either of these values for the Veluwe subsoils. Therefore, the possibilities to measure oxygen *in situ* were studied.

After an ample evaluation of existing methods the oxygen sensor (Oxygen Instruments of Yellow Spring) was selected. Measured values were compared with those obtained by the Winkler titration. In the range 0 - 10 ppm O_2 the meter gave values within $\pm$ 0.2 ppm of the Winkler titration values.

Field measurements.

In a large number of gaugepipes on the Veluwe with filters at different depths the oxygen concentration of the groundwater was measured. As an example, the results for a group of pipes closely together are given in table 5.

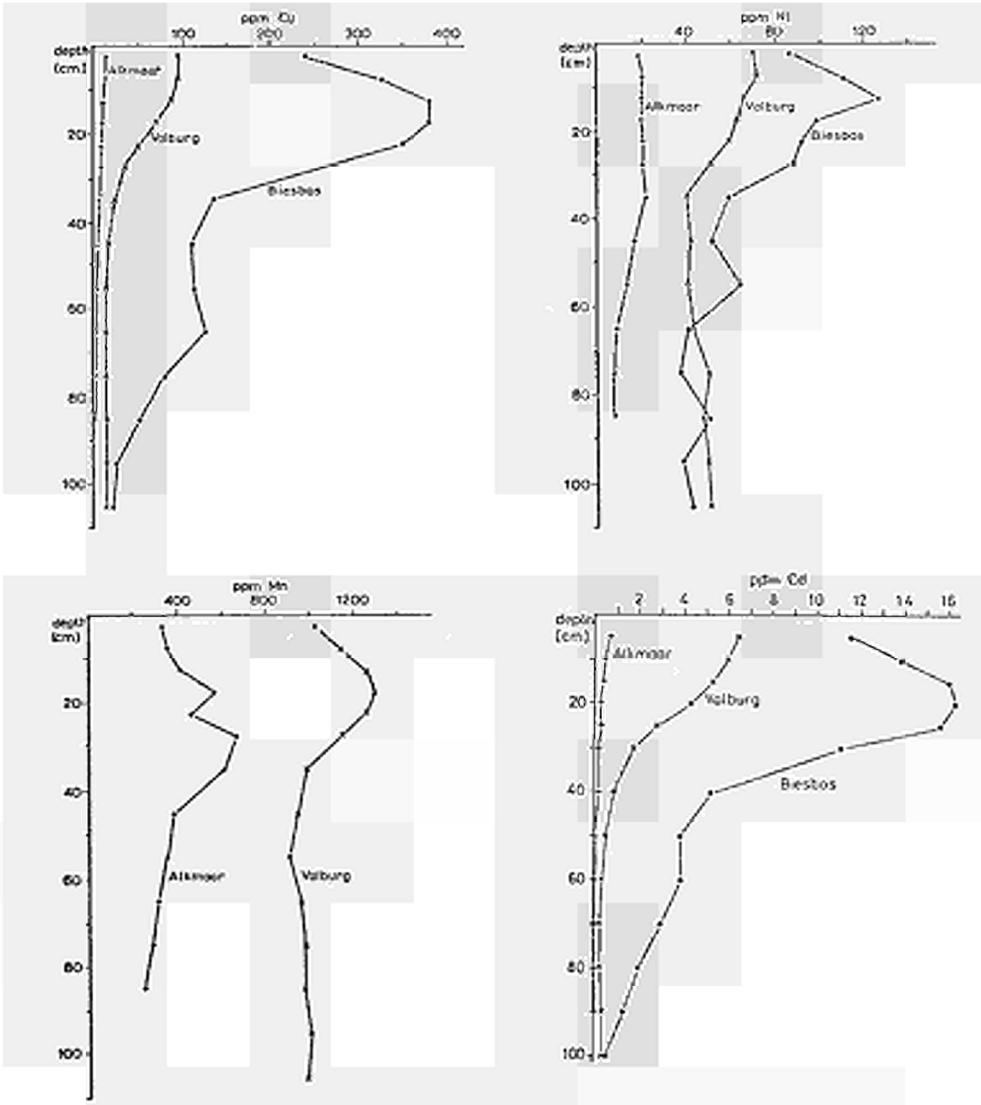


Fig. 1 - Distribution of Cu, Ni, Mn and Cd in various soils.

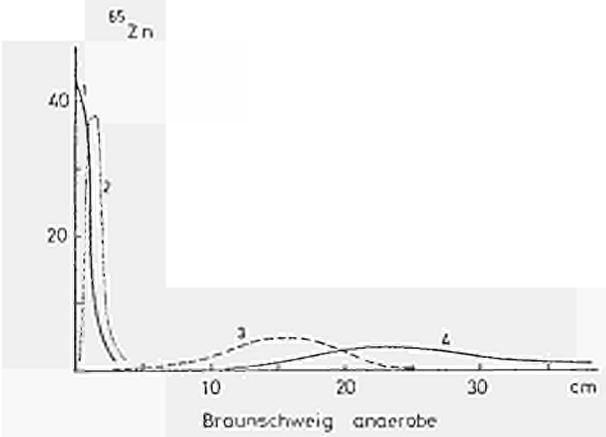


Fig. 2 - Migration of Zn in column with undisturbed soil: Braunschweig sewage field, Br1. Flux artificial soil solution about 1 ml per day. Influent concentration 100 ppm Zn, the first ml were labelled with ^{65}Zn. Curves show position of ^{65}Zn (concentration versus depth). Curves 1, 2, 3 and 4 after resp. 0, 9, 76 and 151 days.

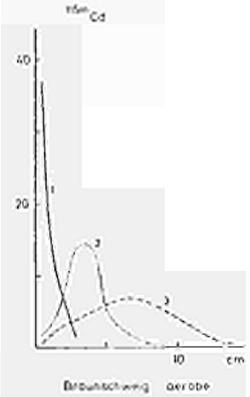


Fig. 3 - Migration of Cd in column with undisturbed soil: Braunschweig, sewage field, Br 24. Flux artificial soil solution about 1 ml per day. Influent concentration 5 ppm Cd, the first ml were labelled with ^{115m}Cd. Curves show position of ^{115m}Cd (concentration versus depth). Curves 1, 2 and 3 after resp. 9 h, 14 and 142 days.

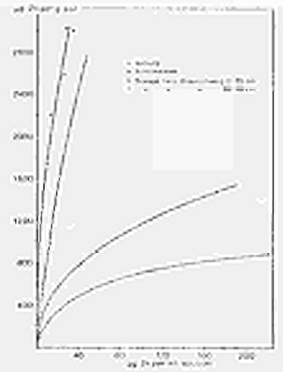


Fig. 4 - Adsorption curves for Zn (Solution: mixture of $CaCl_2$ NaCl and KCl 0.0224 N, Ca:Na:K = 3:1:1).

Table 5 - Oxygen concentrations measured in well R.I.D. 33A - 103.

Depth Filter in meters	O_2 conc. in ppm	temp. in °C	Date
52- 56	9.5	10.0	9-5-1975
52- 56	10.2	10.0	28-5-1975
65- 69	3.0	9.0	9-5-1975
65- 69	2.9	9.0	28-5-1975
87- 91	5.2	9.0	9-5-1975
87- 91	5.2	9.0	28-5-1975
120-123.5	0.6	9.0	9-5-1975
120-123.5	1.0	9.5	28-5-1975
136-140	1.7	9.5	9-5-1975
136-140	1.8	9.5	28-5-1975
181-185	0.6	10.0	9-5-1975
181-185	1.1	10.0	28-5-1975
190-192	3.1	10.0	9-5-1975
190-192	3.9	10.0	28-5-1975

The groundwatertable was approximately 20 m below the soil surface. These borings are situated in the centre of the Veluwe (Kroondomein) where the soils largely consist of stowed pre-glacial sandy materials. This picture of decreasing concentrations of oxygen with depth is also found on other sites of the Veluwe, although lower concentrations (less than 2 ppm) at shallow layers are more common. It can be concluded therefore that the developed device is useful to measure oxygen *in situ* in groundwater. The measurements are acceptable with respect to reproducibility and the errors are with 0.5 ppm of the reading.

The considerable contribution of K. Harmsen of the Dept. Soils and Fertilizers, A.U. Wageningen is thankfully acknowledged.

Publications - 1975.

EL-BASSAM, N., P. POELSTRA and M.J. FRISSEL. Chrom und Quecksilber in einem seit 80 Jahren mit städtischen Abwasser berieselten Boden.
Z. Pflanz. und Bodenk. Heft 3: 309-316 (1975).

FRISSEL, M.J., P. POELSTRA and P. REINIGER. Chromium in soils in "The Behaviour of Chromium in Aquatic and Terrestrial Food Chains". Biology Division, Eur.Comm., Wageningen p. 27-42. (1975).

FRISSEL, M.J. and P. POELSTRA. Fate and effect of inorganic mercury fungicides; a chapter in "Soil Sanitation", D. Mulder (Ed). (1975).

MULDER, D.J., K. HARMSEN, M.J. FRISSEL, P. POELSTRA. Some chemical problems related to the infiltration of purified water of the River Rhine in the Soils of the Veluwe, Netherlands. Report prepared for the ad-hoc committee on geochemistry of the Technical Working group for Artificial Recharge in the Veluwe. Prepared in cooperation with the Lab. of Soils and Fertilizers, Wageningen, and the Government Institute for Water Supply, The Netherlands. External Report 24 (1975).

LEXMOND, TH.J., F.A.M. DE HAAN and M.J. FRISSEL. On the methylation of inorganic mercury and the decomposition of organo-mercury compounds; A review. Accepted by Neth.J.Agr.Sci. (1975).

FRISSEL, M.J. The programme of the Association EURATOM-ITAL. "Benelux-issue" of the Journal of Radioanalytical Chemistry (1975).

FRISSEL, M.J. Development of Agricultural Nuclear Research. Accepted as paper for the Int. Fertilizer Congress" Moscow, June 1976 (1975).

KLUGT, N. VAN DER, P. POELSTRA, E. ZWEMMER. Multielement analysis of soils by computerized instrumental neutron activation analysis. Submitted to J. of Radioanal. Chemistry.

KLUGT, N. VAN DER, P. POELSTRA, P. BOSMAN. Sample preparation for low activity measurements in case of calamities.
ITAL report November 1975 (in Dutch).

Resultaten van het project No. 5

Hoofd van het team en wetenschappelijke medewerkers:

G. Verfaillie, J.-J. Bourgois.

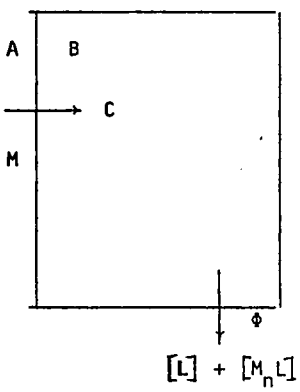
Titel van het project:

Kinetics of uptake of heavy metal-ions by intact plants.

Beschrijving van de resultaten:

1. Theoretical approach.

As pointed out in the report of project No. 6, evidence has been obtained that heavy metals, mainly cadmium, which is considered here because of its interaction with ^{65}Zn, ^{54}Mn, are easily bound to proteic compounds rich in cystein and might be transported as such. Taking into account the molecular weight and composition of a known cadmium binding protein (e.g. CdBP, molecular weights 5000 - 10000), the number of cadmium atoms that can be chelated in such molecules may amount to 4 or 8. On this premise, a theoretical model has been built, which may explain the multiphasic shape of the absorption isotherms observed in the study of the kinetics of root uptake of heavy metal-ions by intact plants. The model may be described as follows:



Let B be an internal compartment of root tissue in contact with the external nutrient medium A in which the concentration of free ions of a heavy metal M is represented by C. The interactions between the n metal-ions and each molecule of an organic ligand L with which they may combine, are represented by:

$$nM + L \underset{k'}{\overset{k}{\rightleftharpoons}} M_nL,$$

with opposed reaction velocities:

$$v = kC^n [L]; \quad v' = k'[M_nL].$$

If the ligand produced in the compartment B at a metabolic rate R_L moves out of it, complexed or not, at a flow rate ϕ, the elimination of the metal-complex M_nL induces a compensatory uptake of free metal ions from the medium A. After reaching steady state conditions, the uptake rate R of heavy metal-ions and the metabolic production rate R_L of the ligand should satisfy the following three equations:

$$R = n(v-v') = nkC^n[L] - nk'[M_nL] \qquad \text{(equ. 1)}$$

$$R = n\phi[M_nL] \qquad \text{(equ. 2)}$$

$$R_L = \phi[L] + \phi[M_nL] \qquad \text{(equ. 3)}$$

Eliminating [L] and $[M_nL]$ from these equations gives:

$$R = \frac{nkR_LC^n}{kC^n + k' + \phi} = \frac{nR_LC^n}{C^n + \beta_n + \phi_k} \qquad \text{(equ. 4)}$$

in which β_n is the gross dissociation constant of the complex M_nL and equal to the ratio k'/k.

This reduces equ. 4 to

$$R = \frac{V_m \cdot C^n}{C^n + K_m^n} \qquad \text{(equ. 5)}$$

The uptake rate calculated according to equ. 5 depends on 3 parameters:

V_m : is the maximum rate of the metal uptake limited by the metabolic rate R_L of the appropriate ligand.

K_m : is an affinity parameter also dependent on the overall metabolism via the flow rate. This parameter represents the concentration of free metal-ions corresponding to half saturation of the produced ligand.

n : is the number of metal-ions that can be chelated by each ligand molecule.

Equation 5 is quite similar to the one introduced by Michaelis and Menten in the kinetics of enzymatic reactions in homogeneous phase conditions, wich has been used by plant physiologists, in working out the carrier theory of ion uptake. In the present case, however, the limiting factor is the rate of production of a biochemical ligand. Moreover, the expression of the uptake rate involves terms of higher power of the concentration. If various ligands L_n are simultaneously acting in the same compartment, the resulting rate for the uptake of metal-ions will be the sum of all partial rates R_n:

$$R = \Sigma R_n = \Sigma \frac{V_{mn} \cdot C^n}{C^n + K_{mn}^n}$$

Only for n = 1 the partial isotherm is really hyperbolic and identical to that of Michaelian kinetics. For all other values of n, the partial isotherms have a sigmoidal shape with a single inflexion point of maximum slope at a concentration of metal ions C_I at which the 2nd derivative of R_n vanishes:

$$\left(\frac{d^2R_n}{dC^2}\right)_I = 0 \longrightarrow C_I = K_{mn}\left(\frac{n-1}{n+1}\right)^{\frac{1}{n}}$$

At these inflexion points, the slopes are becoming steeper and steeper as n increases, and the resulting total isotherm will have a multiphasic pseudo-hyperbolic shape.
Figure 1 represents the absorption isotherms that might be obtained by summing up, in various ways, the partial isotherms corresponding to three ligands having a same production rate and able to complex respectively 1, 4 and 8 metal ions as in the assumed case of CdBP.

2. Chemical interaction between cadmium and cystein.

When an acidified solution of cystein is back titrated with KOH in the presence of cadmium, crystals begin to precipitate at a pH much lower than that required for the precipitation of $Cd(OH)_2$ in the absence of cystein. The crystals redissolve at higher pH without releasing free Cd^{++} ions as it has been controlled with a Cd^{++} ion selective electrode. The pH range of permanent precipitation for a constant total cadmium concentration 10^{-2} M has been determined for various concentrations of total cystein and as shown in fig. 2. The upper boundary of the solid phase is built up of two segments of straight lines having different slopes. The delimitation points A and B have respective abscissae corresponding to Cd/Cys ratios 1 and 2.

Applying a method described by Chanutin (J. Biol. Chem. 143, 737, 1942) and using a Cd^{++} electrode for the determination of the concentration fo free Cd^{++} ions in equilibrium with various total cystein concentrations and pH, the maximum ratio of bound Cd/total Cys seems, indeed, to be equal to 2 (fig. 3).
From these observations it may be infered that each molecule of cystein can complex 1 or 2 cadmium ions.

3. Foliar uptake of zinc and cadmium.

This theoretical approach has been considered, (1) in project No. 6 in relation to root uptake, (2) in preliminary experiments on the kinetics of foliar uptake and further transport to other plantparts (*Phaseolus vulgaris* L., cv "à rames d'Espagne) of Zn (^{65}Zn stable isotope of the same element and Cd because of the possible interaction. Experimental results in this respect are not yet available.

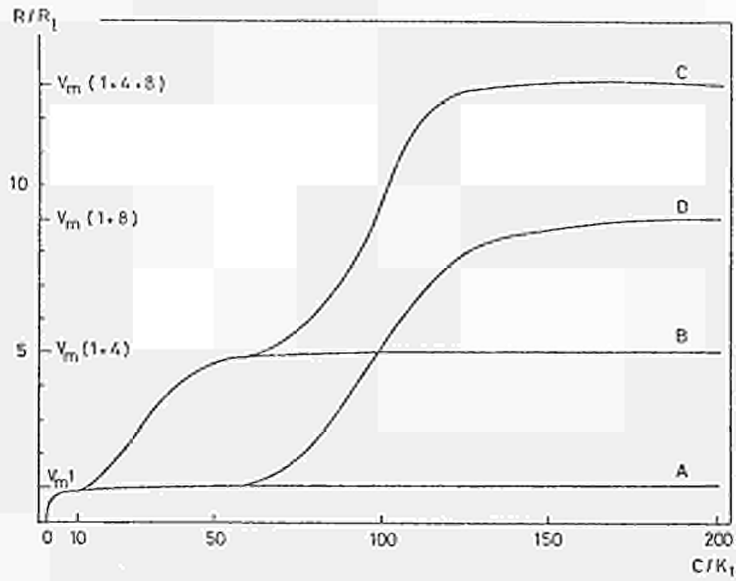


Fig. 1 - Root uptake of heavy metals. Complexation theory.

A, isotherm for n=1
B, isotherm for n=1 and n=4
C, isotherm for n=1, n=4 and n=8
D, isotherm for n=1 and n=8
The arbitrary affinity parameters are: K_1=1, K_4=30 and K_8=100.
The rates of uptake R are normalized on
R_L = 1.

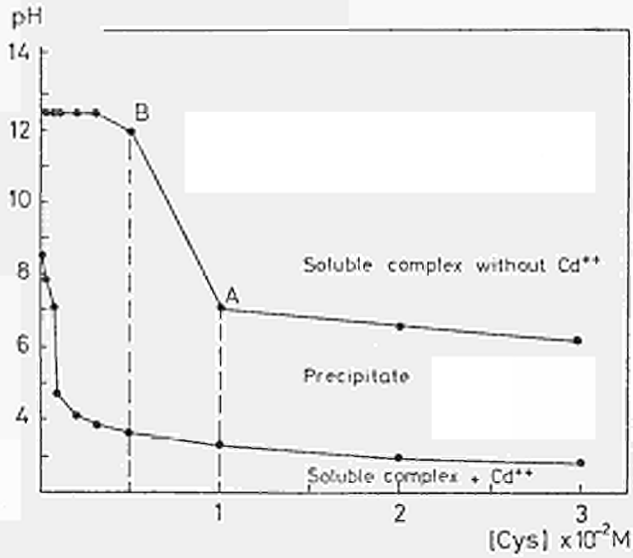


Fig. 2 - Phase diagram of Cd-Cys complexes. The total cadmium concentration is 10^{-2} M.

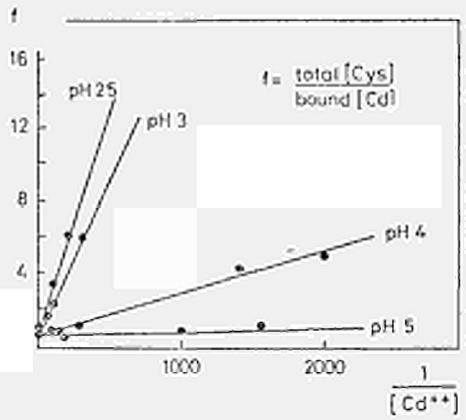


Fig. 3 - Extrapolation plot for determining the maximum possible number of bound Cd atoms at various pH-levels.

Publications - 1975.

VERFAILLIE, G. Nouvelle approche cinétique adaptée à l'étude des échanges gazeux et ioniques chez *Oryza sativa* L. en aquiculture. Ph.D. thesis. Université Catholique de Louvain, (June 1975).

Resultaten van het project No. 6

Hoofd van het team en wetenschappelijke medewerkers:

C. Petit, S. van de Geijn, G. Verfaillie.

Titel van het project:

Transport, accumulation and redistribution of heavy metals in intact plants.

Beschrijving van de resultaten:

1. Study of the mechanism of Cadmium uptake by plant roots.

The study of cadmium uptake is of considerable importance in relation to the problem of ^{65}Zn availability, because of the known interaction of both elements. Part of this research, performed with tomato plants (*Lycopersicon esculentum* Mill. cv. Moneymaker) and described in the annual report 1974, has shown that the cadmium content of plants may, under certain circumstances, increase the rate of the cadmium uptake itself. Working on animal tissues, several scientists have reported that cadmium ingestion stimulates, within a few hours, a "de novo" synthesis of a cadmium binding protein (CdBP). This CdBP apparently is very similar to the metallothionein already described in 1960, namely: a soluble protein, cystein rich (30% of all amino acid residues), high cadmium affinity with possible competition for Zn and other cations (one metal atom for 3 -SH radicals), low molecular weight in the range of 6000 up to 14000. A cadmium binding fraction with a molecular weight of the same order of magnitude was once reported in microorganisms.
Therefore, the existence of a similar induction being suspected in plant material, the root uptake of cadmium has been studied in relation to some of the CdBP properties: amino acid composition, analysed by exchange chromatography, and molecular weight determination with gel chromatography. The following results were obtained thanks to the kind collaboration of E. Marafante, J.M. Mousny, C. Myttenaere from the Biology group at Ispra, Italy, where all measurements have been done.
Tomato plants have been grown for 6 weeks on a complete nutrient solution contaminated either during 5 weeks or during the last 24 hours with three concentrations of cadmium: 0 (control), 5×10^{-7} M and $1{,}5 \times 10^{-5}$ M Cd. According to the treatment, ^{109}Cd and various labelled amino acids were added to the nutrient medium. Labelled fractions, from the root and limb extracts, were separated by gel chromatography (Sephadex G 75).
Results show:

1. a stimulating effect of cadmium on the total cysteic content, in a group of 17 analysed amino acids, which could explain the increased uptake of cadmium at a later stage, considering the high affinity of this metal for the -SH groups.
2. a high cadmium content of the postmicrosomal supernatant of root and limb extracts: between 60 and 70% of the cadmium present in the filtrates of the corresponding homogenates.
3. the association of cadmium, present in the postmicrosomal supernatant with organic compounds of three different molecular weights (MW): $\geq$ 80000, $\simeq$ 12000, $\simeq$ 4400.
4. association of the more than 70% of the cadmium from this supernatant with the 4400 MW fraction. This holds true for both root and leaf tissues (fig. 1, a, b, c). In this fraction, cadmium is probably bound to thiol groups even without the stimulating effect of cadmium, as stated above. This may be infered from the recovery of ^{35}S labelled cystein and cystin, given to the cadmium free control plants during the last 24 hours of their culture (fig. 1, e and f), as compared to the recovery of a mixture of other amino acids labelled with ^{14}C (fig. 1, d). As shown in the figures, cadmium and the cystein compounds are indeed recovered in

chromatographic fractions of equal low molecular weight.

5. associated of the residual 30% of the cadmium from this supernatant with fractions of high molecular weight (MW $\geq$ 80000). This is mainly true for leaf extracts (fig. 1, c) and to a smaller extent, for the root extracts (fig. 1, a and b).
6. particular comportment of cadmium in the roots as compared to the comportment in the leaves.

After a long contamination period, a third peak of Cd shows up. This peak corresponds to an intermediate molecular weight, MW = 12000 (fig. 1b), for which no cysteic compounds are recovered, when isolated from the roots of control plants (fig. 1, e and f). This means that without cadmium supply there is no particular accumulation of cysteic compounds to which the metal could be bound in this 12000 MW fraction. Although, on the basis of the present results and the information concerning animal tissues mentioned above, a "de novo" incorporation of cysteic amino acids induced by cadmium might be expected in this particular fraction of 12000 MW, no experimental data, confirming this hypothesis in plant material, are actually available.

2. *In vivo* measurement of ^{115m}Cd distribution in tomato stems.

In order to allow a detailed interpretation of the total countrate curves, described in the former reports, β-spectrometric methods, developed for ^{45}Ca (project No. 30), have been used for the *in vivo* determination of the distribution of ^{115m}Cd inside the stem of tomato plants. The three following adjacent tissue layers were considered: the most external tissue (0-800 µm), from the xylem up to and including the internal phloem (800-2000 µm), the pith (2000-3500 µm). Several phases in the uptake and redistribution process, which can be distinguished by these *in vivo* measurements, are described below.

Root uptake results in filling of the xylem vessels with the labelled solution in the first 6 - 7 hours. During this period, the redistribution to other parts of the tissue starts. Cadmium, as measured by ^{115m}Cd, moves laterally at a rather slow rate, and reaches the cuticle in about 20 h. This result is confirmed by the total countrate measurements with ^{109}Cd, the electrons of which have a very low penetration power and thus can only be detected when emitted in the proximity of the cuticle.

After 60 hours of Cd uptake from a continuously renewed solution, there is no evidence of a saturation of the inner and outer layers of the stem, whereas the xylem and the surrounding tissue (800-2000 µm) reach somewhat earlier a constant labelling level.

The substitution of the radioactive solution by a normal complete nutrient solution or by a solution of high ionic strength results in a rapid leaching of cadmium from the xylem region, and also from the pith. In the external parts of the stem the cadmium content decreases very slowly. When the nutrient solution is substituted by a solution of low ionic strength (e.g. demineralized water), no leaching or redistribution is observed. Describing the slow lateral movement of cadmium as a diffusion process and considering the tissues as a semi-infinite medium, the boundary of which (the xylem vessels) is kept at a constant concentration, C_o, the following theoretical expression for the concentration, C, as a function of time, t, and distance, x, holds:

$$C = C_o \operatorname{erfc} \frac{x}{2(D.t.)^{\frac{1}{2}}}$$

where D is the apparent diffusion coefficient. According to this relationship, the distance of penetration of any given concentration is proportional to the square root of time. When data obtained from the measurements of the maximum energy of the β-spectra, expressing the distance to the cuticle, are presented in a graph against the square root of time, a good linearity is obtained. The apparent diffusion coefficient shows a value of approximately $5 \times 10^{-8} - 5 \times 10^{-9}$ cm^2/sec.

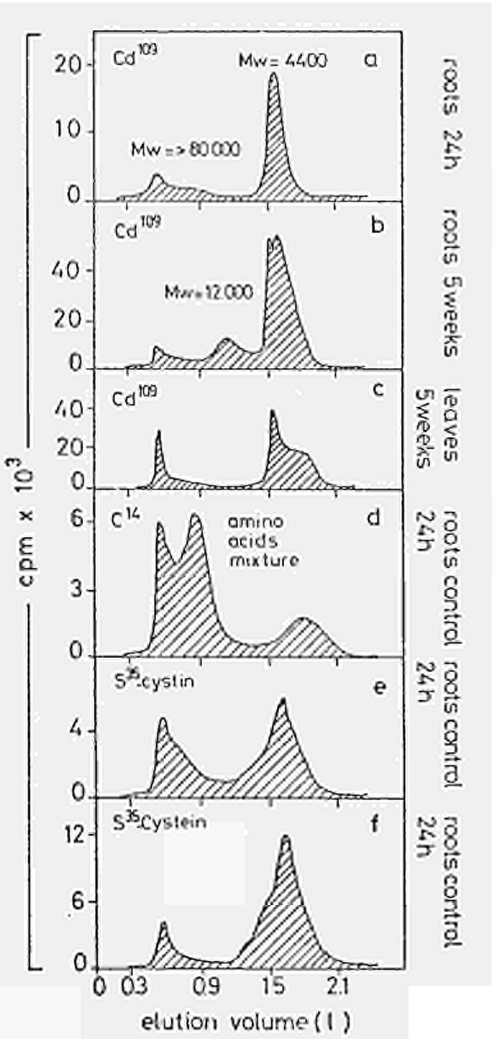


Fig. 1 - a, b, c, d, e, f.

Gell chromatography on Sephadex G-75, applied to post-microsomal supernatant isolated from root and leaf tissues of plants containing ^{109}Cd and various labelled amino acids. All plants were 6 weeks old at the time of sampling. The periods, 24 h and 5 weeks, indicate the application time of the corresponding isotope before harvest. The control did not receive any cadmium, which was supplied to the treated plants at a concentration of $5\ 10^{-7}$ M Cd. The molecular weight (MW) is given for various eluted fractions.

Publications - 1975.

GEIJN, S.C. VAN DE, Ch. PETIT. A non-destructive determination of the internal distribution of cadmium (^{115m}Cd) in the stem of a tomatoplant (*Lycopersicon esculentum*, Mill. cv. Moneymaker) by the analysis of the externally measured beta-spectrum. ESNA Newsletter on the application of nuclear methods in biology and agriculture. No. 5, December, 1975.

Resultaten van het project No. 7

Hoofd van het team en wetenschappelijke medewerkers:

G. Desmet, M. van Duyvendijk-Matteoli.

Titel van het project:

Uptake and release of heavy metals by subcellular structures, mainly chloroplasts and mitochondria.

Beschrijving van de resultaten:

Research in this project aims at collecting data concerning the regulating effect of the existing plant micronutrient (e.g. Mn, Zn) and macronutrient (e.g. Mg, K) content on the further accumulation of e.g. ^{65}Zn, ^{54}Mn and the related metal Cd. Mainly two aspects of this problem have been considered this year:
(a) the interaction between Zn, Mn and Cd.
(b) further study of the possible mechanism governing the mineral element exchanges at the chloroplast level.

a. The influence of the interaction between Cd, Mn, Zn on their individual content in plant leaves. Spinach plants (*Spinacea oleracea* L. cv. Verbeterd Breedblad) are grown on a continuously flowing fresh nutrient solution. In one experimental approach plants are transferred during 48 hours from this system to a medium containing 8.10^{-6} M $Cd(NO_3)_2$.
The Mn, Zn, and Cd content of these Cd treated plants has been determined by AAS and compared with the one of non-treated plants. The plants used for this determination were respectively 10, 15, 20 and 28 days old. Although plants were gorwn in a climate controlled room on nutrient solution of constant concentration, variability of the results excluded conclusions concerning the effect of the age of plants.
On the average, the 8.10^{-6} M $Cd(NO_3)_2$ treatment during 48 hours decreases both the Mn and Zn content of the aerial part of the spinach plants by approx. 30%. This decrease might suggest that the growth in dry weight of the aerial parts of the plants is less affected by Cd^{++} than the uptake of Mn^{++} and Zn^{++}. A dilution of Mn and Zn would be the result. In order to control this explanation, growth curves of Cd-treated and non-treated plants are defined now.
In another experimental approach, spinach plants are grown on nutrient solutions containing 1/4, 1/2, 1/1, and 2/1 of the usual Zn concentration i.e. 10^{-6} M, while all the other mineral concentrations are left unchanged. The aim of this experiment is to study whether plants containing different amounts of Zn in their aerial parts show quantitatively a different pattern of interaction with $Cd(NO_3)_2$, compared to the one mentioned above. Data are not yet available.

b. Effects of Cd on the ion exchanges in isolated chloroplasts.
Beside the fact that Cd inhibits the electron transport in the thylakoid membranes of isolated chloroplasts at the water-splitting site (cfr. publication B.B.A.) Cd has been found to uncouple the electron transport i.e. to deteriorate the energy conservation (ATP formation) of these membranes. The source of energy necessary to produce ATP is built up with the formation of a membrane potential. It arises from a charge separation, followed by a movement of H^+, K^+, Mg^{++} and Cl^- ions through the thylakoids creating a proton concentration gradient supposed to be the driving force for ATP synthesis. Being aware of these phenomena, a study was started to see whether Cd can participate in ion exchanges in chloroplasts, and thus whether its content in these organelles is different in light and dark. A first approach of this problem is made by measuring the H^+ exchanges in isolated chloroplasts. These H^+ concentration changes have, of course, to be measured at low buffer

capacity of the medium, i.e. in the presence of a weak TES buffer (8.10^{-4} M instead of 5.10^{-2} M). No definite results are obtained. In order to be sure about the effect of the change in the composition of the medium on the rate of electron transport, again measurements of the influence of Cd on the rate of O_2 production have been done. It became obvious less Cd is necessary to deteriorate the energy metabolism, when the amount of TES is decreased. Moreover, the uncoupling action of Cd is strongly enhanced (fig. 1). Further experiments are necessary in order to fully understand these observations, which are additional to those published previously.

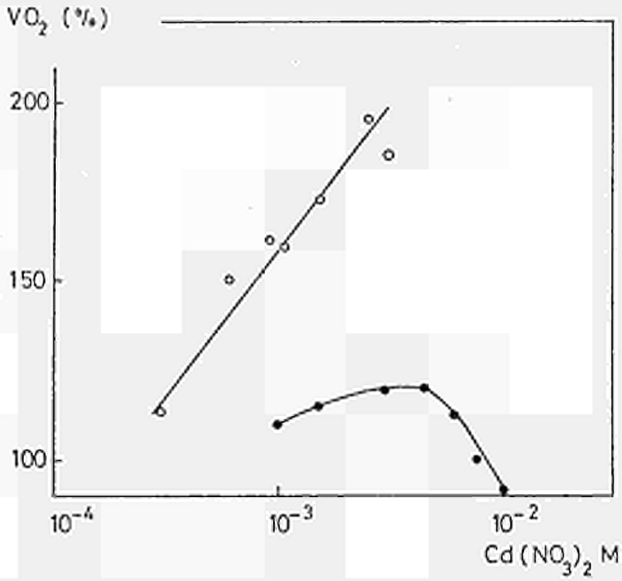


Fig. 1 - Rate of electron transport (O_2 production) at different Cd^{++} concentrations, in the presence of two buffer concentrations.
●—● 5.10^{-2} M TES pH 7.6
○—○ 8.10^{-4} M TES pH 7.6

Publications - 1975.

DUYVENDIJK-MATTEOLI, M.A. VAN and G. DESMET. On the inhibitory action of cadmium on the donor side of photosystem II in isolated chloroplasts. Biochim. Biophys. Acta, 408 (1975) 164-169.

DESMET, G., A. DE RUYTER and A. RINGOET. Absorption and metabolism of CrO_4^{2-} by isolated chloroplasts. Phytochemistry vol. 14, 2585-2588 (1975).

Resultaten van het project No. 8

Hoofd van het team en wetenschappelijke medewerkers:

K.H. Chadwick, H.P. Leenhouts, K.J. Puite.

Titel van het project:

Primary effects of radiation in inert and biological material.

Beschrijving van de resultaten:

Analysis of genetic effects in the spidermite (*Tetranychus urticae* Koch. (Acarina: Tetranychidae).

Results on the hatchability of haploid and diploid eggs of the spidermite following X-ray and fast neutron irradiation arising from the research programme carried out by A.M. Feldmann have been analysed using the molecular theory.

If $N = \alpha D + \beta D^2$ is the mean number of DNA double strand breaks per cell after a dose (D) and P_h and P_d are the probabilities that a double strand break leads to mortality in the haploid and diploid eggs, respectively, then the hatchability of the haploid and diploid eggs is given by:

$$S_h = \exp(-P_h N); \quad S_d = \exp(-P_d N).$$

Thus a relationship should exist between the haploid and diploid hatch, such that:

$$\frac{\ln S_h}{\ln S_d} = \frac{P_h}{P_d} = k,$$

which is independent of the way in which the DNA double strand breaks are induced i.e. independent of the radiation used.

It is further assumed that a proportion (w) of the N double strand breaks is transmitted to the following generation, then the hatchability of eggs from the second generation (F_2) is

$$S_h^1 = \exp(-P_h wN); \quad S_d^1 = \exp(-P_d wN)$$

and

$$\frac{\ln S_h^1}{\ln S_d^1} = \frac{P_h}{P_d} = k,$$

i.e. the same relationship should exist between the hatchability of the haploid and diploid eggs in the first and second generations. In figure 1 the logarithm of the diploid hatch has been plotted against the logarithm of the haploid hatch using experimental points derived from F_1 and F_2 hatches following irradiation of the females with X-rays and fast neutrons plus the F_2 hatches following irradiation of the males with X-rays and fast neutrons. The results are compatible with a straight line through the origin as predicted, the slope of which gives $k = 1.52$.

Further development of the theoretical model.

Consideration has been given to formulating a better relationship between the physical interaction process occurring when an ionizing particle passes through water and the radiobiological effect on cell survival which is related to the induction of DNA double strang breaks, according to the molecular theory.

LET effects on cell survival.

The molecular theory proposes that the dependence of the coefficinets $p\alpha$ and $p\beta$, which are used to describe the cell survival curve, on radiation quality can be explained by the different efficiencies for the induction of DNA double strand breaks by the different radiation types.

Starting from the radiation track model, which was already described in the Annual Raport 1974, and which gives the spatial distribution of primary radiation events (ionizations and excitations) in water, firstly the coefficient $p\alpha$, associated with the number of DNA double strand breaks created in the passage of one ionizing particle, was calculated. The following assumptions were made:

a. a DNA strand break occurs when a water radical can diffuse to the DNA strand (effective diffusion length parameter σ);

b. the number of water radicals, produced by an ionization or excitation event and, effective in strand breakage are F_{ion}, F_{exc}, resp.

c. the geometry of the DNA molecules is taken into account: the average distance between the two strands is 12 Å; each base pair is 3.4 Å long; the number of base pairs per cell is N.

d. the values calculated for a particular radiation type are averages determined for all primary and secondary charged particles participating in the energy deposition.

Using this calculation model it was possible to explain the dependence of $p\alpha$ on various radiation types for T_1 human kidney cells, lymphocytes and chlorella algae cells under aerobic conditions. Figure 2 shows the result for the T_1 cells. In all cases considered it turned out that the water radicals must be created within 5 - 10 Å from the strands in order to be effective in this type of strand break production.
Consideration of the $p\beta$ coefficient, associated with the production of DNA double strand breaks by the combination of two single strand breaks, each created by different ionizing particles, leads to the proposition of a different mechanism for the production of the second break. Following a single strand break, the DNA helix becomes more vulnerable to the production of a second break in the opposite strand induced by a water radical which diffuses over a distance in the order of magnitude of 60 - 100 Å.
This proposition could be in line with findings of radiochemists, who also consider that different processes are involved in the α and β terms.

Somatic mutations.

In view of the recent trend in scientific literature to associate mutagenic and carcinogenic agents the previous theoretical work on somatic mutations in plants has been provisionally extended to a consideration of the dose relationship for radiation induced malignancy.
It has been proposed that the factor which controls the malignant nature of a cell behaves as a recessive genetic character. This means that some diploid cells may carry the recessive malignant character which is prevented from expressing itself by the normal dominant homologous gene, whilst other cells do not carry the malignant factor at all. A somatic mutation in the normal dominant homologous gene of a diploid cell which carries the malignant character may allow the expression of the recessive malignant character. Assuming, on the basis of the molecular theory, that radiation induced DNA double strand breaks can cause somatic mutations or chromosome aberrations, a mathematical equation can be derived to provide a general description of the dose response for radiation induced malignancy which is nonlinear and peaked for cells which carry the recessive malignant character.

$$C = K(1 - \exp(-q(\alpha D+\beta D^2))) \exp(-p(\alpha D+\beta D^2)).$$

Consideration of this equation and the mechanism of DNA double strand breakage by radiation leads to the prediction that the incidence of malignancy will be lower following a low dose rate exposure of sparsely ionizing radiation and that densely ionizing radiation will be more effective per rad and exhibit a relatively small dose rate effect.

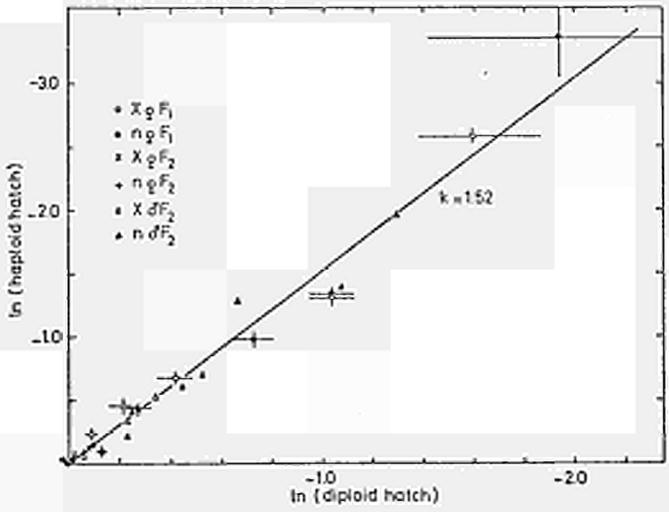


Fig. 1 - The relationship between the hatchability of haploid and diploid eggs of the spider mite in two successive generations following irradiation with X-rays and fast neutrons.

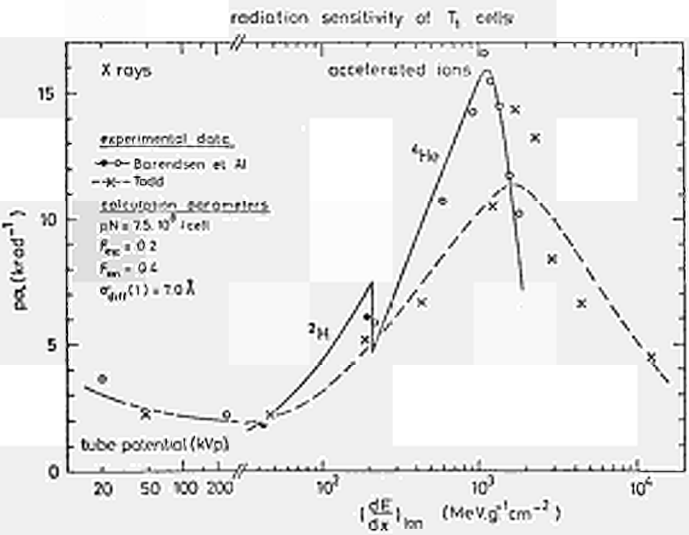


Fig. 2 - Comparison of the calculated and experimental values of pa for T1 kidney cells irradiated with different radiation types in aerobic conditions.

Publications - 1975.

LEENHOUTS, H.P. and K.H. CHADWICK. Stopping power and the radiobiological effect of electrons, gamma rays and ions. 5th Microdosimetry Symposium, Italy, 1975 (in press).

LEENHOUTS, H.P. and K.H. CHADWICK. An analysis of radiation induced damage in the spider mite: the relationship between mortality of haploid and diploid eggs in two successive generations. IAEA Symposium on Biological Effects of Low level Radiation pertinent to protection of man and his environment. Chicago 1975 (in press).

LEENHOUTS, H.P. and K.H. CHADWICK. Radiation induced malignancy: a recessive somatic mutation? Implications for radiological protection. 3rd European IRPA congress. Amsterdam 1975. Condensed Papers A7.

Resultaten van het project No. 9

Hoofd van het team en wetenschappelijke medewerkers:

P.A.Th.J. Werry, A.W. Spanjers, K.J. Puite, F.M. Engels, F.A. Hoekstra.

Titel van het project:

Irradiation dose-mutation relation in rad dose range.

Beschrijving van de resultaten:

A. Chromosome aberrations in root tips of *Haplopappus gracilis* (Nutt) Gray.

1. The Giemsa staining-technique, as reported in the annual report 1974, showed a good longitudinal differentiation in the chromosomes. However, since we are particularly interested in possible involvement of the telomeres in radiation-induced chromosome rearrangements, modification of the technique was continued. At last a procedure was developed - a modified Giemsa-C-banding technique - which produces an excellently suitable staining. Figure 1a shows a photograph of a stained metaphase and in figure 1b the karyogramme of *H. gracilis* is schematically represented. The banding pattern of the chromosomes permits the individual identification of each chromosome as even the homologous chromosomes stain in a different way. Chromosome 1A presents an ideal subject because it has one band, in the form of two points at the very end of its p-arm. Therefore we concentrated on analyzing all the chromosome aberrations which involve chromosome 1A.

2. To see whether or not there is some synchrony in the root-meristem cells resulting in an oscillating frequency of mitotic cells, the number of mitoses in root tips was determined at regular intervals. These experiments revealed that the mitotic index is very constant at a value of 9%. So, with respect to cell division, the root meristem of *H. gracilis* can be regarded as a homogeneous population under the cultivation conditions used.
   Since at this stage of investigations we were only interested in chromosome aberratons, experiments were carried out to determine the optimum time interval between the irradiation and the fixation of the root tips. It was found that, 24 h after irradiation those cells were in metaphase that were irradiated in G1 phase. Therefore the root tips were fixed 25.5 h after irradiation with a 0.05 colchicine treatment during the last 1.5 h.
   This final treatment of the roots with 0.05% colchicine dissolved in culture medium resulted on the one hand in a considerable accumulation of metaphases. On the other hand chromosomes were more sticky, and therefore it was more difficult to produce well-spread metaphases. Nevertheless the colchicine treatment was maintained.

3. Root tips were irradiated in water with X-rays (250 kVp, 15 mA, HVL 1.9 m Cu) at doses of 950 and 1425 rad resp. Chromosome aberrations induced by this treatment were studied and examples of found rearrangements involving the p-arm of chromosome 1A are shown in figures 2a, b, c. In figures 2b, c, d, drawn interpretations of the rearrangements are presented. These illustrations very clearly show rearrangements with the telomere of the p-arm of the chromosome involved in the formation of the aberration.
   Up till now - the number of investigated cells amounting to approximately 10.000 - we have found no reciprocal translocations between the p-arm of chromosome 1A and any other arm. Only simple translocations to the p-arm of chromosome 1A were found. Cell dicentrics between that arm and any other chromosome show the telomere-band at the point of connection. We may conclude that - at least in *Haplopappus gracilis* - the involvement of the telomere in the formation of radiation-induced chromosome aberrations is no rare event.

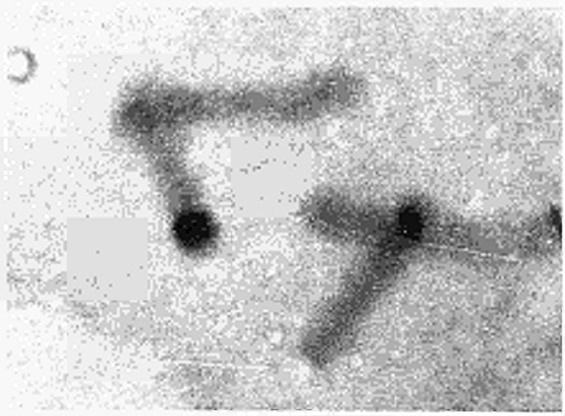
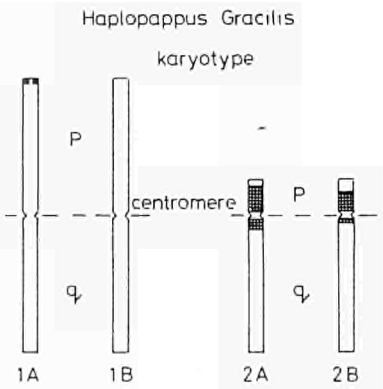


Fig. 1 - a. Metaphase plate of *Haplopappus gracilis* (Nutt.) Gray. The telomere band of chromosome 1A is clearly shown (arrow) (3000 x)



b. Karyogramme of *Haplopappus gracilis* according to the metaphase-plate shown in a.

Fig. 2a

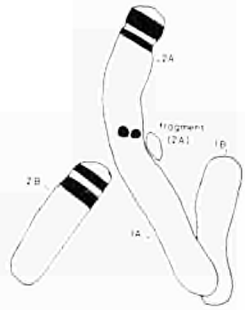


Fig. 2b

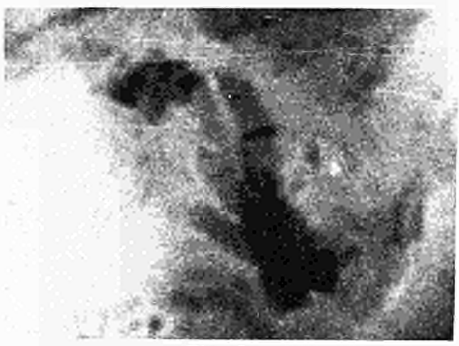


Fig. 2c

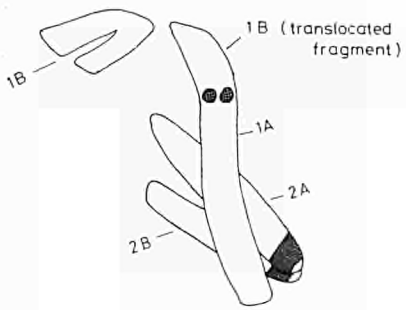


Fig. 2d

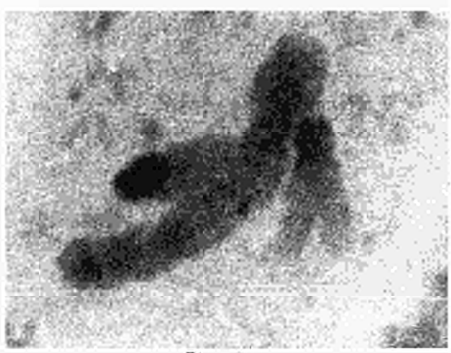


Fig. 2e

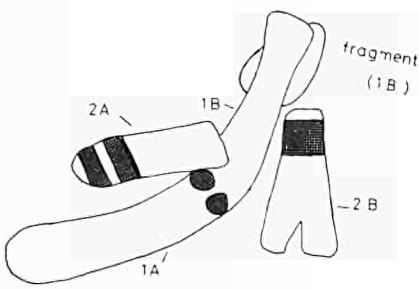


Fig. 2f

Fig. 2 - Radiation induced chromosome aberrations involving chromosome 1A in *Haplopappus gracilis* (Nutt.) Gray.
a: dicentric between chromosome 1A and 2A.
c: translocation to chromosome 1A from chromosome 2A.
e: dicentric between chromosome 1A and 1B.
b, d, f: drawn interpretations of respectively a, c and e.

B. Cell suspension cultures of *Haplopappus gracilis* (Nutt.) Gray.

1. Growth characteristics (increase in dry and fresh weight) of a "home made" cell line have been established and with respect to that our culture is comparable to cultures described in literature.

2. Since not all the cells in the suspension cultures are really single, experiments are currently going on to produce real single cell-suspensions. Results obtained so far indicate that addition of cell wall degrading enzymes to the suspension culture results in a considerable increase of single cells without influencing the growth characteristics.

C. Irradiation effects on the survival of different pollen species.

As a basis for the study of the effect of irradiation upon the survival of different pollen species the metabolic conditions and some synthetic capacities have been studied in collaboration with G.M. Desmet.

Mitochondria, isolated from ungerminated but respiring pollen of *Typha latifolia* L. cannot phosphorylate, in contrast to mitochondria isolated from pollen, germinated for three hours *in vitro* (Annual Report 1974). A suitable indicator for the characterization of the developmental condition of the Electron Transport Chain (ETC) was thought to be the ratio

$$\frac{V\ O_2\ DNP}{V\ O_2\ oligomycin}$$

The uncoupler DNP causes an important stimulation of O_2-uptake in the case of NADH-oxidation, always higher than the maximum stimulation by ADP, whereas oligomycin inhibits all oxidative phosphorylations and related O_2-uptake.

The ratio $\frac{V\ O_2\ DNP}{V\ O_2\ oligomycin}$ resembles the normal respiratory control ratio, $\frac{V\ O_2\ state\ 3}{V\ O_2\ state\ 4}$, with the advantage of being much less sensitive to slight differences in isolation procedure and endogenous enzyme activities such as ATP-ase and coupling factors. As can be seen in fig. 3, mitochondria from germinating pollen of *Typha* and *Nicotiana* clearly show an increase in the ratio from the onset of germination, indicating the ETC to undergo a rapid development. In contrast, ungerminated pollen of Aster and *Tradescantia* contain mitochondria, already equipped with a well developed ETC. Fig. 3 also shows that the ETC reaches top capacity at about 75 min, 30 min and approximately from the onset of germination for *Typha*, *Nicotiana* and both *Tradescantia* and *Aster*, respectively. These periods strikingly coincide with the so called lag-time of germination *in vitro*, which means the time that elapses prior to emergence of the pollen tubes, namely 70, 35, 5 and 4 minutes for *Typha*, *Nicotiana*, *Tradescantia* and *Aster*, respectively. It is suggested, that the lag-time is an indication of the developmental condition of mitochondria in the ungerminated fresh pollen grain.

In order to tackle the problem, whether indeed oxidative phosphorylation is absent in fresh respiring *Typha* and *Nicotiana* pollen, a direct measurement of ATP has been performed. The method of ATP measurement with firefly extract and a scintillation counter has been studied and described in an external report to be published in 1976. ATP measurements of the oligomycin-inhibited *Typha* pollen during respiration in humid air confirmed the absence of oxidative phosphorylation under these conditions since the ATP concentration remained constant during the incubation period, whereas pollen of *Aster* and other Composites showed a rapid decrease in ATP content, indicating a high turnover (see fig. 4). Quantitatively, this turnover amounted to, at least, approx. 1900 nmoles of ATP/mg x h on the average, as could be calculated from gaschromatography data of the oligomycin-inhibited O_2-uptake.

Calculations on the Adenylate Energy Charge (AEC = $\frac{ATP + 0.5\ ADP}{ATP + ADP + AMP}$) of pollen, respiring in humid air gives a direct parameter of the balance between ATP-generating and ATP-utilizing sequences. All Composite pollen had an AEC of about 0.9, indicating the respiratory system to be in balance with the energy demanding systems.
The AEC of respiring *Nicotiana* and *Tradescantia* pollen was as low as 0.65, which means that synthetic processes prevail and ATP-generating processes are limiting. Foregoing illustrates the extremely divergent stages of development, in which bi- and trinucleate pollen dehisce.
One of the energy demanding processes during respiration in humid conditions is protein synthesis, as has been confirmed by incorporation experiments.
In viable respiring *Aster* pollen the incorporation of L- ^{3}H leucine into proteins ceased after approx. 2.5 h, although respiration remained constant for at least the duration of the same period. It is suggested, that the abrupt decline in the incorporation activity, probably depending on a rapid breakdown of the protein synthesizing machinery, is closely related to the rapid loss of vitality in trinucleate Composite pollen.

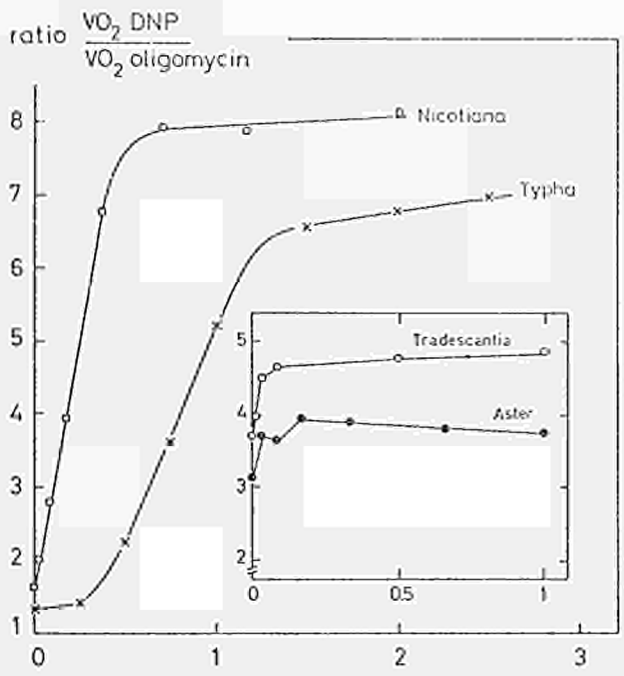


Fig. 3 - Ratio $\frac{VO_2\ DNP}{VO_2\ oligomycin}$, as an indicator of the developmental stage of the mitochondrial Electron Transport Chain during germination *in vitro* of 4 different pollen species. The O_2-uptake of the mitochondrial isolations was measured polarographically at 24°C, NADH serving as substrate. Oligomycin was added in a concentration of 6 µg/ml, DNP up to maximum stimulation of O_2-uptake (up to about $5.\ 10^{-4}$ M).

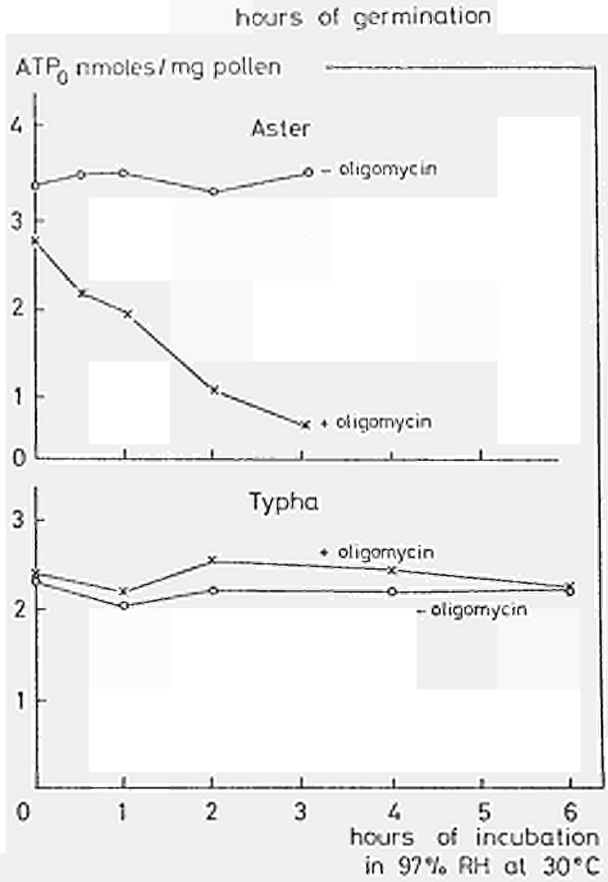


Fig. 4 - Effect of oligomycin treatment upon the ATP content of *Typha latifolia* and *Aster tripolium* pollen during respiration in humid air (97% RH) at 30°C.

Publications - 1975.

SPANJERS, A.W., F.M. ENGELS, P.A.Th.J. WERRY, K.H. CHADWICK, H.P. LEENHOUTS. New evidence on chromosome joining after radiation. Proceedings V Symposium Microdosimetry, Verbania Pallanza. In press.

HOEKSTRA, F.A. and J. BRUINSMA. Respiration and vitality of binucleate and trinucleate pollen. Physiologia Plantarum 34, 221-225 (1975).

HOEKSTRA, F.A. and J. BRUINSMA. Viability of *Compositae* pollen: Germination *in vitro* and influences of climatic conditions during dehiscence. Zeitschrift für Pflanzenphysiologie 76, 36-43 (1975).

Resultaten van het project No. 10

Hoofd van het team en wetenscnappelijke medewerkers:

K.H. Chadwick and K.J. Puite.

Titel van het project:

Applied dosimetry.

Beschrijving van de resultaten:

A. Lyoluminescence studies.

Last year a start has been made with a study of the lyoluminescent properties of various saccharides. When these sugars, after being irradiated, are dissolved in water a light signal (lyoluminescent (LL) signal) can be observed. The near tissue equivalency of the saccharides offers perspectives to apply them for dosimetric use in low energy X-ray fields and fast neutron beams. The following properties have now been studied:

a. the lyoluminescent sensitivity of various saccharides.

Materials have been purchased from Baker Chemical Company, BDH Chemicals, Difco Laboratories and Merck. The relative LL signals obtained are:

mannose,	Baker	100 and 52	xylose,	Baker	3
	BDH	86		Difco	2 and 5
	Difco	86		Merck	2
	Merck	14			
trehalose,	Baker	1	glucose monohydrate,		
	BDH	1		Baker	20
	Merck	3		BDH	6
				Merck	8

b. the fading of the lyoluminescent signal.

The fading of mannose samples stored in the dark in 1.8 mm thick polyethylene capsules at ambient temperatures of 23 to 26 °C has been measured up to 5 months after irradiation. When the LL signal is plotted versus the logarithm of time after exposure a straight line is obtained. Normally, the LL signal is read 48 h after exposure to avoid the initial fading. The fading during the first week following these 48 h amounts to 4.5%.

c. the LL response of mannose to 250 kV X-rays, fission neutrons and 15.5 MeV neutrons.

The dose response curve of mannose from 100 rad up to 100 krad for 250 kV X-rays (HVL 1.9 mm Cu), fission neutrons and 15.5 MeV neutrons has been measured. The curves are supralinear. The 15.5 MeV neutron irradiation has been carried out at the Radiobiological Institute at Rijswijk in cooperation with Dr. J.J. Broerse. The gamma contamination of both (fission and 15.5 MeV neutron) beams was 5%.
The kerma ratio of mannose (6.7% hydrogen content) and ICRU tissue (10.2% hydrogen) depends on neutron energy and is 0.69 for fission neutrons and 0.80 for 15.5 MeV neutrons. Thus, the efficiency of fission and 15.5 MeV neutrons relative to 250 kV X-rays is expected to be at most 69 and 80%. The measured values are 31 and 65%, respectively, indicating an effect of the LET of the neutrons. The measurements will be extended to 5 MeV neutrons. From the data obtained up till now it is clear that mannose can be used for dosimetric measurements in fast neutron fields. Measurements in low and medium energy X-ray fields are planned in future.

B. Measurement of dose and radiation "quality" of X-rays with an HVL of 0.1 to 3 mm Cu.

Intercomparison of absorbed doses and other relevant radiation parameters are of great value for the evaluation of biological and physical results obtained at various centers. Work has been carried out to measure absorbed dose and radiation quality in low and medium energy X-ray fields using thermoluminescent (TL) materials. The results are directly applicable in intercomparison programmes e.g. of the European Late Effect Project Group (EULEP) and of the International Atomic Energy Agency (IAEA). The IAEA foresees an extension of its cobalt-60 dose intercomparison service to include also conventional deep therapy X-ray machines.
Two different systems have been developed.
(1) For medium energy X-rays (HVL above 1.1 mm Cu) measurements should be performed at 5 cm depth in water using CaF_2 and LiF TL powder in normal (3 mm inner diameter) capsules.
(2) For low energy X-rays (HVL between 0.13 and 1.4 mm Cu) measurements should be carried out at 2 and 10 cm depth in water using LiF TL powder in normal capsules. In the first system information on radiation quality can be derived from the ratio of the CaF_2 and LiF TL responses.
In the second system use is made of the strong attenuation of low energy X-rays in water. The radiation quality can be derived from the ratio of the two LiF readings.
The uncertainty in the absorbed dose value with these systems amounts to $\pm$ 4%. The uncertainty in the determination of the radiation quality of the incident beam is $\pm$ 3 to $\pm$ 7%, depending on energy.

C. Perspex Dosimeters.

Measurements have been made to study the effect of different post-irradiation storage temperatures on the time course of the induced OD change at 305 and 314 nm in three different batches of 1 mm HX dosimetry perspex. Irradiation of samples of batches 2, 3 and 4 to 2.5 Mrad was carried out at 23°C at $\sim$ 800 krad/h, after irradiation samples were stored in air at 20°C, 35°C and 45°C. OD measurements made at different times after irradiation were carried out at 20°C. Fig. 1 presents the results from which the following conclusions can be drawn:

1. the three batches of supposedly identical dosimetry perspex are not identical.
2. the relative increase in OD at 305 nm after irradiation is batch and temperature dependent.
3. the time at which the maximum OD at 305 nm is achieved is temperature dependent.
4. the oxygen diffusion induced fading is temperature dependent, and also batch dependent.

Thus, after a short calibration irradiation at a normal temperature the OD-dose calibration curve determined at 305 nm will depend on the storage temperature after irradiation and on the time at which measurement is made, e.g. 24 h as is recommended by the suppliers of HX perspex, or in the OD maximum, and differences of up to 10% in measured curves may arise. This means that the calibration curve issued by the suppliers of HX perspex is not absolute. At temperatures below 35°C the OD-dose relationship at 314 nm will show smaller temperature and time variations in the period 1 - 25 h after irradiation. The rapid fading of OD at 35°C in batch 4 indicates that 1 mm samples of this material cannot be considered a satisfactory dosimeter, 2 mm thick material would improve the time stability of all batches.

Measurements of calibration curves for all three batches at 23°C at ∿ 800 krad/h from 1.5 - 3.5 Mrad have confirmed these conclusions. Further measurements will consider the effect of different temperatures and dose rates during irradiation on the response of the dosimeter.

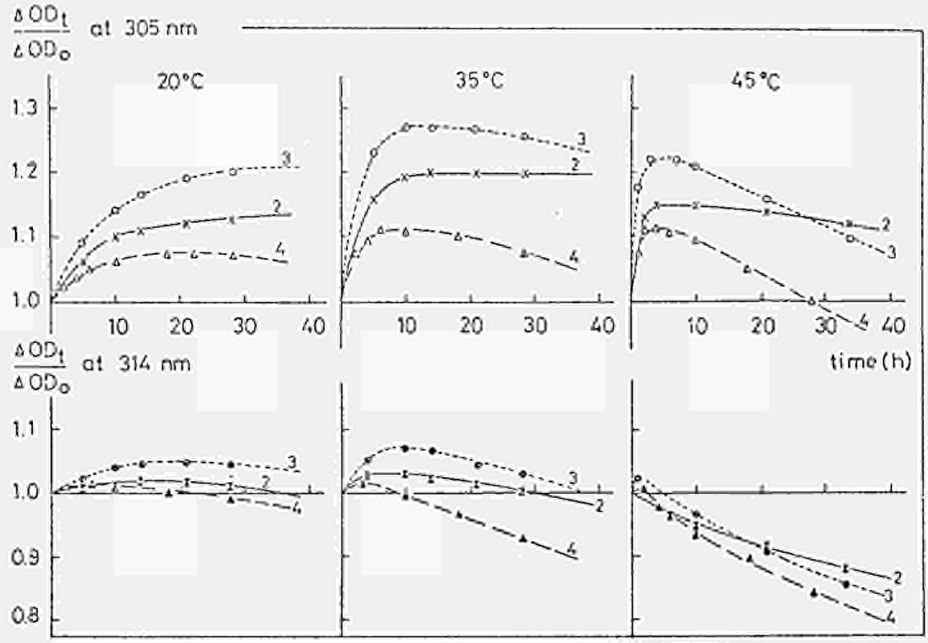


Fig. 1 - The storage temperature-time dependence of the OD of 1 mm samples of batch 2, 3 and 4 HX perspex after irradiation at 23°C to 2.5 Mrad at 0.87 Mrad.h^{-1}. Measurement at 305 and 314 nm.

Publications - 1975

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Contrat N°: SC 010/094-72-1 BIA N
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List of scientists having contributed to this report :

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Thème général du contrat : Movement of soil ions and their uptake by plants.

Project Nr.1 : Ion Movement and Exchange in Soils.

The research work on the simulation of salt movement through soils has been continued this year by solving some problems involved in the numerical calculations of the movement of unreacting solutes.
The first aim was to dispose of an easy tool for characterizing the soil properties affecting these movements. When dealing with constant water fluxes through soil columns of finite length, analytical solutions can be relatively easily developed which give the time-concentration relationship as a function of only two parameters : the porosity (Φ) of the material used and the "Peclet number", the latter includes besides experimental flow rate and column length, the hydrodynamic dispersion coefficient(D). According to analytical equations developed by Brenner (Chem.Eng. Sci.17,229-243,1962), a Fortran program was developed which gives the best "Φ - D" pair characterizing the observed flow of solute through an experimental material. It is based on a least square method the principle of which can be briefly summarized as follows.

Porosity and dispersion coefficient are first roughly estimated from the collected volume corresponding to a relative concentration of 0.5 and from the slope of the breakthrough curve at that point. Afterwards the computer chooses a range of Φ and D around their approximate values. In these intervals two best fit curves are calculated, the one describes the Φ values best fitting the experimental breakthrough curve for some arbitrary values of D and the other describes the best fit D values for some arbitrary values of Φ. The best "Φ - D" pair is obviously given by the intersection of those two curves.

The CPU time required by the computer for such a calculation is rather low; by instance for a flow experiment involving some twenty concentration-time (or collected volume) pairs, the best fit porosity and dispersion coefficient are calculated with a relative precision of $\pm$ 0.0001 within only 10 to 20 seconds on an IBM 370-158 computer.

This program was tested on a set of experimental breakthrough curves of tritiated water and chloride-36. Its efficiency was found to be excellent in spite of the diversity of used materials (from clay soils to sand or even glass beads) and in spite of the diversity of column lengths and flow rates. In all cases good agreement was observed between the experimental breakthrough curves and those simulated by introducing the Φ and D parameters in the analytical equations. An interesting fact is that such a simulation procedure applies as well to completely saturated soils as to unsaturated soils provided of course that water flux remains constant during the flow experiment. In this last case the air volume trapped in the column is regarded by solutes as participating to the inaccessible solid fraction. Independent measurements of average water contents agreed fairly well with the porosity values calculated by the program.

If on the one hand analytical equations are very useful in determining the Φ and D parameters with the highest precision and if on the other hand they are suitable for modeling the movement of unreacting solutes, they however lend themselves with difficulty to further introduction of additional terms describing, by instance, ion exchange or precipitation phenomena. Therefore it was necessary to develop some numerical solutions of the partial differential equation including both convection and diffusion driving forces. It has been previously done using a simple finite difference method. Those solutions have given interesting practical results with reasonable accuracy for field agronomists, but their general applicability was restricted.
Attempts were made this year to develop another numerical solution which is not limited by such restrictions, but which could easily handle source and sink terms.
Its convergence and stability were tested.
Parallel to these studies on salt movements, more fundamental work was carried out on sodium-calcium soils and clays. A statistical study on the Na-Ca exchange properties of soils has been undertaken. Exchange isotherms were established on several soils at various ionic strengths; a first set of 27 isotherms is still being interpreted.
The changes of particle size and shape during the Na-Ca exchange were also studied . Such properties obviously affect the flow behavior of water and solutes through soils. Four methods were used to this purpose : viscosity measurements, light scattering, electron microscopy and X-ray diffraction. It was found that the aggregation status of clay plates remained roughly constant for adsorbed calcium percentages lower than about 60 or 70%.

For higher calcium loading a sharp increase in aggregation was observed resulting in larger dimensions and more spherical shape of particles. It is easily understood that such changes of structural properties will greatly improve the permeability of Ca-rich soils.

Referring to the possible sink and source mechanisms satisfactory results have been obtained in modelling ammonium oxidation by mixed cultures, taking account of substrate concentration, temperature, pH, O_2 partial pressure and substrate inhibition.

A mathematical model which describes the growth and the activity of Nitrosomonas and Nitrobacter in a well-aerated mixed culture, has been developed. It was assumed that the molar growth yield is constant and that the Monod and the Michaelis-Menten kinetics obtained, with respect to growth and oxidation rates, respectively.

Independent determinations of kinetic constants were used as a basis for preliminary digital computer simulations which, at both at 20 and 30°C showed a good fit of the theoretical curves to experimental data.

Simulation studies, at different temperatures, predicted that the nitrite peak would vanish at decreasing temperatures.

This agrees with the present observations and also with other results obtained with river water.

A mathematical model which describes the effect of pH and dissolved oxygen on growth and activity of Nitrosomonas and Nitrobacter in mixed culture has been developed.

A good fit was obtained between experimental data and theoretical curves computed with parameters estimated by independent methods.

Experimental data together with simulation studies show that inadequate aeration induces a temporal shift of ammonium and nitrite oxidations resulting in a transient nitrite accumulation similar to that caused by an increase of temperature.

Project Nr.2 : Uptake of Solutes by Plants from a Dilute Environment.

Our previous studies on the uptake of NH_4^+, K^+ and Mg^{2+} by intact soybean roots indicated that the uptake isotherm followed the single, multiphasic pattern of uptake when the uptake was considered over a wide range of substrate concentration. This prompted us to study the kinetics of phosphate and calcium uptake by using ^{32}P and ^{45}Ca respectively.
The uptake of $3.22 \times 10^{-7} - 3.22 \times 10^{-3}$M of phosphate is mediated by phase I in the range $3.22 \times 10^{-7} - 6.45 \times 10^{-6}$M, by phase 2 in the range $1.61 \times 10^{-5} - 1.29 \times 10^{-4}$M, and by phase 3 in the range $1.29 \times 10^{-4} - 3.22 \times 10^{-3}$M. The discontinuity of uptake between $6.45 \times 10^{-6} - 1.61 \times 10^{-5}$M is accompanied by a marked jump and the transition occured at about 8.59×10^{-6}M, and 1.29×10^{-4}M was chosen as the transition point between phase 2 and 3. The sharpness of the inflection point and the linearity of the double reciprocal plots suggested the clear demarkation of individual phases. The kinetic constants of each phase increased in a regular manner while the affinity(1/Km) decreased gradually.
Similarly the uptake of calcium could be represented by a biphasic isotherm in the concentration range $6.2 \times 10^{-6} - 1.25 \times 10^{-3}$M.
The effect of different concentrations of phosphate and calcium on the growth response and ion accumulation was also studied. Maximum growth of soybean occured in the concentration range of 129 - 161 µM of P and 124 - 249 µM of calcium respectively. This study indicated that the accumulation of P and Ca in the soybean plants followed the same trend as that of uptake.
An experiment was carried out on 25 day old maize plants, on the uptake of manganese, Mn concentration being varied from 0.005 to 3 ppm. The results were calculated according to a Michaelis kinetic with the following parameters :

Vm for first step (microequivalents/h.g.DW^{-1})	7,8305
Vm_2 for second step	33.453,50
Km_1 (microequivalents)	0,9730
Km_2	1.960,45

We have also calculated the relevant parameters in Thellier's formulation with the following results :

$2.3 \frac{A}{r}$	amp.gr.P.S.$^{-1}$	= 10,6669 $\pm$ 1,4471.10^{-7}
r	ohms	= 6591,9433
$\frac{B}{(\overline{P})}$	microequi.$^{-1}$	= 10,1643
$LOG \frac{B}{(\overline{P})}$		= 1,0071
m		= 7,3611 $\pm$ 0,3330
A(25°C)		= 3,0572.10^{-3}
T		= 13,4497

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Contractor : Université Catholique de Louvain
Contract n° : 096-72-1-BIO B
Head of the Research Team : A. Goffeau
Title of Project : Transport of radionuclides by biological membranes.

Substantial progresses have been made in 1975 for the two research projects.
1. Among the three mitochondrial NADH dehydrogenases, the site of action of the stimulation by strontium and calcium of the oxidation of NADH by isolated mitochondria has been further pinpointed to the enzyme located at the external face of the inner mitochondrial membrane. New experimental evidences permit to propose a physiological function for this stimulation, described so far in vitro only. New informations are provided which strengthen the conclusion that strontium and calcium act primarily by formation of a complex with NADH, which exhibit increased reactivity as substrate for enzymatic as well as for chemical oxidations.

2. The cellular uptake of strontium and calcium by intact yeast cells has been further characterized. It is now clear that calcium and strontium are driven actively into the cell by an electrochemical potential build up by a plasma membrane ATPase. The latter has been extensively studied in particular for its interactions with divalent cations. It is concluded that this ATPase is not directly involved as calcium or strontium pump. Its main role seems to build an electrochemical potential by active extrusion of protons. The understanding of this mechanism has permitted the demonstration of a large variety of inhibitory and stimulatory conditions for both the cellular entry and the exit of strontium or calcium. In particular, these studies have demonstrated an unknown ionophoretic property of the antibiotic Dio-9 which stimulates proton/potassium and calcium/potassium exchanges. In addition, the mechanism for control of cellular membrane permeability by cyclic AMP has been further explored by the demonstration and characterization of high affinty binding sites of the hormone to the external cell membrane.

Project 1
Head of the Project : A. GOFFEAU
Scientific staff : M. BRIQUET, A.M. COLSON, M.F. LABAILLE, HA QUOC BAO
Title : Transport of radionuclides by isolated yeast mitochondria

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That the stimulation by strontium and calcium of the oxidation of external NADH by isolated intact yeast mitochondria is related to the transport of the cation is definitively excluded since similar stimulations have now been demonstrated in solubilized NADH-$Fe(CN)_6^{3-}$ oxidoreductase preparations obtained from sonicated inner mitochondrial membranes. This fact makes very likely that the stimulation of the oxidation of NADH obtained with intact mitochondria (the inner membranes of which are impermeable to NADH) is the result of activation of the NADH dehydrogenase located, in yeast and in plants, on the external side of the inner mitochondrial membrane.
The slow chemical, non-enzymatic, reduction of ferricyanide by NADH was also found to be stimulated by strontium and calcium. The latter observation supports the conclusion that the cations reacts with the negative phosphate group of the substrate NADH and forms a pyridine-nucleotide-cation complexe. Such complexes have solubility and redox properties different from those of free NADH.
Two observations throw a new light on the possible physiological meaning of these stimulations. In the absence of cations, the optimal pH of the NADH dehydrogenase is pH 5.0 which is curiously low since the internal pH of yeast was estimated to be 6.5. However in the presence of physiological concentrations of calicum (or strontium) of 5 mM the optimal pH is shifted to the more physiological value of 6.0. The role of the endogenous cellular cations might thus to shift the optimal pH of the enzyme to more physiological values. We also found that ATP exerts a stimulatory action on the NADH dehydrogenase. Since in vitro the stimulation by the cations cancels that by ATP it might be thought that, in vivo, the cations could prevent the effects of a decrease in the cytosolic ATP concentration.
No new crucial informations on the effects of strontium and calcium have been obtained this year by the specific use of respiratory-deficient mutants, the understanding of which presents some difficulties for the moment. We still believe that the genetic approach will be of considerable

value in the future and it must also be pointed out that the obtention of the above data and their interpretations have been greatly facilitated by the mitochondrial expertise maintained in our laboratory for the study of these membrane-deficient mutants.

Project 2
Head of the project : A. GOFFEAU
Scientific staff : F. FOURY, J. DELHEZ, M. BOUTRY and J.P. DUFOUR
Title : Role of the plasma membrane in the transport of radionuclides

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In resting cells of the fission yeast Schizosaccharomyces pombe, the uptake of calcium is stimulated by the addition of 90 mM glucose in the absence of respiration and inhibited by antimycin A in the absence of exogenous carbon source. This uptake requires thus fermentative or respiratory metabolic energy. The calcium uptake by S. pombe exhibits saturation kinetics and high affinity for calcium. At pH 4.5, the apparent Km is 45 µM Ca^{2+} but is not modified by the addition of glucose. Five hundred µM of other divalent cations exert competitive inhibitions of calcium uptake in the following order of affinities : $Sr^{2+} > Co^{2+} > Mn^{2+} > Mg^{2+}$. Inhibition by KCl is also observed but is of mixed competitive/non-competitive type and requires high concentrations of the order of 50 mM. The calcium uptake is temperature and pH-dependent. At 30°C, the uptake rate is at least 10 times higher at pH 8.25 than at pH 4.0. An extrusion of $^{45}Ca^{2+}$, the rate of which is estimated to be lower than one fifth of the uptake, is observed in the presence of glucose only when the external pH is acid. In the absence of respiration inhibited by antimycin A, low concentrations of lanthanum chloride, ruthenium red and hexamine cobaltichloride are inhibitory at external pH 4.5 and stimulatory at pH 8.25 for the uptake of calcium by the yeast cells. In presence of antimycin A, the uncouplers : NaN_3, dinitrophenol and concentrations of carbonylcyanide m-chlorophenylhydrazone higher than 80 µM inhibit the calcium uptake by glycolysing cells at external pH 4.5. Stimulation of calcium uptake by low concentrations of carbonylcyanide m-chlorophenylhydrazone is observed at both external pH 4.5 and pH 7.0. In the absence of respiration, the ATPase inhibitors : N,N'-dicyclohexylcarbodiimide and Dio-9 enhance severalfod the uptake of calcium and elicit a rapid outflow of K^+ into the external medium. It is concluded that Schizosaccharomyces pombe possess an energy-dependent and mediated-transport system for calcium. In the absence of respiration, this transport is affected by glycoproteins reagents, by uncouplers and ATPase inhibitors. The participation of a Dio-9 sensitive plasma membrane ATPase in active transport is further supported by the observation that

both the active cellular uptake of L-leucine and the ATPase activity of a purified plasma membrane fraction were 50 per cent inhibited by 5 to 10 µg Dio-9 per ml. The active cellular extrusion of protons induced by the metabolization of glucose was inhibited by Dio-9. Simultaneously, an outward movement of potassium was induced. At external pH 4.5, Dio-9 elicited a potassium/proton exchange movement down the concentration gradient of both ions. Uncouplers, which alone, induced only a slow linear potassium/proton exchange, increased the velocity of the potassium/proton exchange induced by Dio-9. High external K^+ concentrations reversed the Dio-9 induced proton influx. Classical potassium ionophores were unable to simulate the effects of Dio-9. Our results are consistent with the hypothesis derived from Mitchell's chemiosmotic concepts that the active uptake of calcium and other metabolites is carried out at the expense of an electrochemical potential created by the coupling of ATP hydrolysis to outward proton translocation by a plasma membrane ATPase. The collapse of the membrane potential by Dio-9 or other agents induces proton/potassium or calcium/potassium exchanges.

The stimulation of active transport by 3',5'-cyclic AMP discovered last year in Schizosaccharomyces pombe has been further investigated by characterization of the binding of the nucleotide to a purified particulate fraction containing plasma membranes. After isopycnic centrifugation of this particulate fraction in a sucrose gradient, the cyclic AMP binding activity exhibits a mimodal distribution at density 1.21 g per cm^3 associated to a plasma membrane pH 6.0 ATPase-containing fraction, and density 1.25 g per cm^3 associated to a ribosome-containing fraction. No binding activity is associated with the mitochondrial fraction equilibrating at density 1.19 g per cm^3. The binding activity of the purified plasma membrane fraction exhibits a dissociation constant for cyclic AMP of 3 nM and is competitively inhibited by cyclic GMP. The Scatchard plot for binding of cyclic AMP to the plasma membrane fraction indicates the presence of only one class of binding site. The plasma-membrane binding activities from the plasma membrane can be partially solubilized by alcaline deoxycholate and separated into three peaks by elution from a DEAE-cellulose column.

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M. BOUTRY, F. FOURY and A. GOFFEAU
(submitted for publication)

- Plasma membrane adenosine triphosphatase and active cellular uptake in the yeast Schizosaccharomyces pombe. Inhibition by Dio-9
  F. FOURY and A. GOFFEAU
  (submitted for publication)
- Membrane-bingind sites for adenosine 3':5'-monophosphate in the yeast Schizosaccharomyces pombe
  F. FOURY
  (submitted for publication)
- Stimulation by divalent cations of NADH dehydrogenase activity in yeast mitochondria
  A. SOUCHAY, HA QUOC BAO, J. MATTOON and A. GOFFEAU
  (in preparation)

Contractor: United Kingdom Atomic Energy Authority

Contract No.: 133-74-1 B10 UK

Head of research team: Mr. A. Morgan

General subject of contract: Uptake of tritium from accelerator targets

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The evolution of tritium from another used 5 Ci neutron generator target has been studied. Measurements were commenced within two hours of the last bombardment of the target and continued for eighty-five days using the methods described in the previous report.

Tritium/titanium particles were produced from used and unused generator targets by mechanical shock using the apparatus described previously and also by ultrasonic agitation in water. The activity distribution of particles, produced by mechanical shock from an unused target, was studied. Leaching experiments in water were carried out with particles from used and unused targets to determine the amount of tritium removed.

Tritium absorption by human subjects following exposure to particulate tritium by both ingestion and application of particulates to the skin was investigated. The experiments were carried out using particles produced by ultrasonic means and also by mechanical shock. The body content of tritium was determined by urine analysis.

Results of Project

Head of Project and scientific staff: J.D. Eakins
A.E. Lally

Title of Project: Internal contamination with tritium arising from the use of tritium-titanium targets in neutron generators

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The evolution of tritiated water and tritium gas from the target is shown in Fig. 1. Tritium was evolved at a mean rate of 70 μCi h^{-1} 2 hours after bombardment. This decreased rapidly to 7 μCi h^{-1} after 1 day and to 3 μCi h^{-1} after 1 week. The ratio of tritiated water to tritium gas was 100:1 initially and increased slowly to 500:1 by day 50.

The activity distribution on particles produced from an unused 5 Ci target by mechanical shock was measured with the cascade centripeter. The results obtained in four experiments are given in Table I expressed as a percentage of the total activity collected.

Table I

Activity distribution of particles from an unused tritium/titanium target

Run No.	Mean aerodynamic diameter			
	>12.5 μm	12.5-4.0 μm	4.0-1.5 μm	<1.5 μm
1	99.17	0.79	0.03	0.01
2	88.82	0.03	0.03	11.12
3	83.50	16.03	0.08	0.39
4	70.63	16.71	11.40	1.24
Mean	85.53	8.39	2.90	3.19

On average 8% of the activity was associated with respirable (< 5 μm aerodynamic diameter) particles. The count median diameter (CMD) of the particles produced was 5 μm (σg = 2).

Particles produced from targets attached to an ultrasonic probe and immersed in water were smaller than those produced by mechanical shock,

having a CMD of 2.4 μm and σ_g of 1.5. In addition to tritium in particulate form, activity was also present in solution as HTO. The amount of tritiated water produced was dependent upon the time elapsed since the target was bombarded. A target bombarded 4 hours earlier gave a ratio of HTO/particulate tritium of 1.35 but 24 hours later the ratio had dropped to 0.23.

Tritium was leached very slowly from particles suspended in water. The leaching rate over 5 days was 10^{-3}% day^{-1} for particles from a used target and 2×10^{-5}% day^{-1} from an unused target.

To study absorption, particles generated ultrasonically from a target, which had been bombarded 5 hours previously, were used. One subject drank a suspension of particles (50 μCi) in water and a similar activity was applied to 50 cm^2 of the skin of the inside forearm of a second subject and left in contact for 4 hours. Urine samples were collected from both subjects for 4 days following administration and analysed for tritium. No activity was detected in any of the samples. As it was possible that tritiated water, associated with the particles, was being removed while they were in suspension, a second study was carried out in which dry particles, produced by mechanical shock from a target bombarded 5 hours previously, were used. 26 μCi were ingested and 80 μCi applied to the skin as before, but again no tritium was detected in urine. It is considered that an absorption of 1% would have been detected by this means. These results indicate that the radiological hazard resulting from either ingestion or percutaneous absorption of tritium in particulate form is negligible.

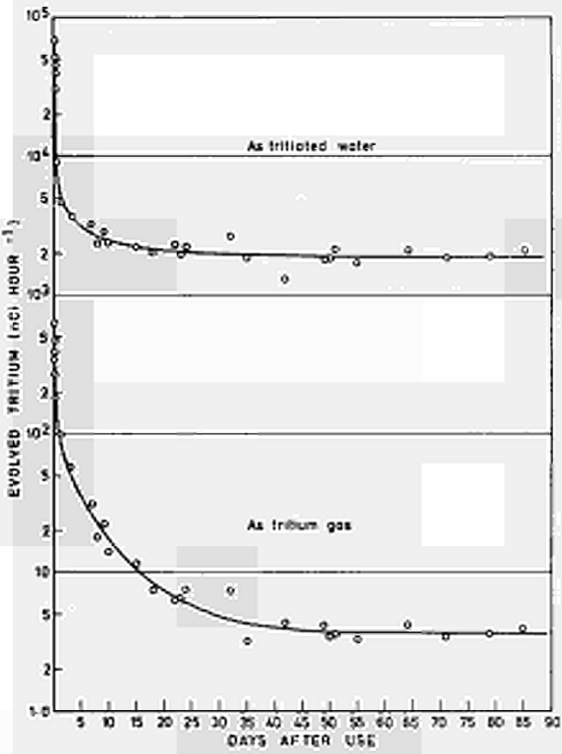

Fig. 1. Evolution of tritium from a tritium/titanium target bombarded 2 hours prior to the first measurement.

Contractor: Ministry of Agriculture, Fisheries and Food
Fisheries Radiobiological Laboratory, Lowestoft
Contract No: 137-74-7 BIO UK
Head of research team: Dr N.T.Mitchell
General subject of contract: Environmental behaviour of radioactive wastes

Project 1. Transport and distribution of transuranio radionuclides in the marine environment following waste disposal from fuel reprooessing.

This group of radionuclides have a special relevance in public health terms because of a combination of high radiotoxicity and long radioactive half-life. Studies have been based on controlled discharges from Windscale into the Irish Sea. Initially, effort was concentrated on plutonium, study of which was already under way before the contract began, studies of americium being added at a later stage.

The underlying aim of this research has been to add to our understanding of the environmental behaviour of these radionuclides. The first stage has to be an evaluation of their distribution in the environment and samples have been taken representative of the important compartments of the environment - water, sediment and biota. Water has been sampled from research vessels of the Ministry and analysis made for both suspended matter and filtrate. Seabed sediment has been collected by grabbing and coring. Representative biota include various species of fish and shellfish as well as algae.

Project 2. Transport and distribution of fission-product radionuclides in the marine environment following waste disposal from fuel reprocessing.

The emphasis within this project has been placed on the radionuclides of caesium (-134 and -137). This project has been coordinated closely with transuranic project with some joint sampling programmes. Due to their environmental persistence, relatively large quantities in the discharge from Windscale into the Irish Sea and a modest concentration factor in fish and shellfish, these nuclides are not only important in a public health context but provide a basis for following the dilution and dispersion of wastes over long distances on the continental shelf margins.

The removal mechanism of caesium onto sediment has a special importance since sediment contamination provides a direct pathway to public radiation exposure, one aspect of its environmental behaviour which has been given attention.

Results of Project No. 1. Contract 137-74-7 BIO UK

Joint Heads of Project: D F Jefferies, J A Hetherington

Other Scientific Staff: A K Steele, M B Lovett, D C Denoon

Transport and distribution of transuranic radionuclides in the marine environment following waste disposal from fuel reprocessing

Initial studies have concentrated on plutonium for which an established analytical method was already available utilising ion-exchange purification and electrodeposition of the source prior to counting by use of a silicon barrier layer detector. Alpha spectrometry was employed to measure simultaneously plutonium-239/240 and plutonium-238, and also to separate plutonium-236 added for yield tracer purposes.

The analytical method has been further developed to include the measurement of americium-241 which is separated from the plutonium in the ion-exchange process. This is then purified by a further anion exchange step, involving a heated column and 4M ammonium thiocyanate medium, which provides adequate separation and yield. The same electrodeposition process is employed as for plutonium followed by alpha spectrometry to separate and measure the americium-241 and americium-243, the latter being added for yield tracing.

A large fraction of the plutonium released in liquid wastes through the pipeline from Windscale into the Irish Sea finds its way quickly onto seabed sediment and a similar situation has been found for americium. The distribution of these radionuclides, both their partition between these two primary phases and the relationship between suspended matter and sea water has been explored, most of the samples being collected during cruises using the Ministry's research vessels RV CIROLANA and RV CORELLA. Concentrations in water have been plotted on grids throughout the Irish Sea and northwards up the western coast of the Scottish mainland through The Minch to Cape Wrath. This suggests that the small fraction of activity not removed by sediment stays in the water mass, concentrations being little further modified except by dispersion.

The most important biota in a public health context are fish and shellfish. Both plutonium and americium are found in seaweeds but insignificant amounts of these materials are processed into foodstuffs. However, like certain molluscan shellfish they provide useful indicator systems and for this reason the distribution with distance from source has been

examined and further work instituted by collection of representative water samples so that concentration factor data may be derived. Samples of each of the more commercially important species of fish have been analysed - Plaice (Pleuronectes platessa), Dab (Limanda limanda), Whiting (Gadus merlangus) and Herring (Clupea harengus). Concentrations in their edible flesh is generally low indicating a small concentration factor, probably in the range 1-10. However, concentrations measured are liable to variation, due, it is thought, to the extent to which the filleted flesh is free from fragments of bone and other parts of the fish which would not normally be eaten. Distribution within organs is being undertaken, one notable conclusion of which is that the inclusion or otherwise of skin is a significant factor.

Alongside these studies samples of shellfish have also been examined for plutonium and americium - especially Crab (Cancer peragus), Norway lobster (Norvegicus norvegicus), Mussel (Mytilus edulis) and Winkle (Littorina littorea).

The third and in some ways the most important compartment of the marine environment is sediment, for it contains most of the plutonium and americium discharged from Windscale. In consequence it is important to understand the mechanism by which uptake occurs and especially whether uptake is a reversible process. Cores have been analysed to determine the depth distribution. Nuclide ratios, particularly those involving plutonium-238, are especially useful in dating the sediment core and a further technique which is being pursued is to analyse the interstitial water from the core.

Publication, part of which was supported by this contract:
J A Hetherington, D F Jefferies, N T Mitchell, R J Pentreath and D S Woodhead

'Environmental and public health consequences of the controlled disposal of transuranic elements to the marine environment'.

Presented at the joint USERDA/IAEA symposium on 'Transuranic Elements in the Environment' held at San Francisco, November 1975; to be published by IAEA in 1976.

Results of Project No. 2. Contract 137-74-7 BIO UK

Joint Heads of Project: D F Jefferies, J A Hetherington
Other Scientific Staff: A K Steele, K O Firman, D C Denoon

Transport and distribution of fission product radionuclides in the marine environment following waste disposal from fuel reprocessing

A considerable body of information has been built up by the laboratory from studies in the Irish Sea, though mainly concerning the less conservative nuclides such as zirconium-95/niobium-95, ruthenium-106 and cerium-144. Attention has been turned towards those which exhibit a largely conservative behaviour, as discharges of them have increased to a level which not only facilitates measurement but demands closer attention as a result of their public health significance.

Established analytical techniques have been used measuring the activity by gamma spectrometry following chemical purification in the case of seawater samples by use of ammonium molybdophosphate supported on silica gel or potassium hexacyano cobalt ferrate. Water measurements have been made following the fate of radiocaesium discharged from Windscale for considerable distances from the point of release. The main route is northwards through the North Channel from the Irish Sea and thence round the Scottish mainland and into the North Sea. Following of its further dispersion has been facilitated by the higher rate of recent discharges and is providing information on circulation patterns in the Northern North Sea, which is of interest to other members of the Community and in this context the laboratory is collaborating with the Deutsches Hydrographisches Institut, Hamburg.

Along with caesium-137 are smaller quantities of caesium-134). The substantial difference in their half-lives (30 and 2.2 years respectively), coupled with the fact that the latter is of the same order as the transit times of water masses involved, makes it possible to use the caesium-137: caesium-134 ratio to estimate these times.

Work with biota has centred mainly on fish since this is the seafood with the greatest public health significance. Other biota sampled include shellfish and seaweeds, useful indicators which integrate short-term fluctuations.

The laboratory has long had a substantial interest in the sediment compartment of the marine environment. Though the behaviour of caesium is

regarded as predominantly conservative a significant fraction of the activity is associated with sediment, enough to generate a significant contribution to external exposure of the public at certain points along the local coastline. The spatial distribution has been investigated, principally examining those locations where the sediment is made up of fine particulate material. Vertical distribution in the sediment is also important to determine the mechanism by which accumulation occurs.

With a nuclide of half-life as long as caesium-137 it is important to know the stability of activity taken onto sediment. Surface adsorption plays an important part and is often responsible for most of the uptake. This has been investigated by both field and laboratory experiments, the latter using caesium-134 to measure distribution coefficients and establish adsorption isotherms. The effect of sediment particle size and other parameters, such as concentration of activity in the liquid phase and its salinity, has also been studied. A fundamental question which we are attempting to answer is what happens to the site at which a radioactive caesium atom has been bound after it decays to its daughter product barium.

GENETISCHE STRAHLENWIRKUNGEN

HEREDITARY EFFECTS OF RADIATION

EFFETS HEREDITAIRES DES RAYONNEMENTS

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Jahresberichten beschrieben:

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

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

095-BIOB CEN Mol (Maisin)

094-BIAN ITAL, Wageningen (De Zeeuw)

Biology Group Ispra

Contractor: Professor K. A. Marcker, Department of Molecular Biology, University of Aarhus,
Contract No.: 122-74-1-Bio DK
Head of research team(s): Dr. Ole Westergaard
General subject of Contract: Molecular Mechanisms for DNA Repair/DNA Replication

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INTRODUCTION

The lack of mutants in DNA synthesis among eukaryotic cells has resulted in a major gap in our understanding of the correlation between replication and repair of the eukaryotic chromosome. Various attempts have been made to overcome this gap. Thus, the enzymes involved in the DNA metabolism have been intensively studied and their activity and intracellular localization correlated with the physiological conditions of the cell. Another approach has been to study the various intermediates in the DNA metabolism in order to get a detailed picture of the individual steps in replication and repair.

We have for some time been involved in studies of the DNA metabolism in Tetrahymena pyriformis and shall in the following describe how damage to DNA causes accumulation of both a particular DNA polymerase (i) and intermediates in the DNA synthesis (ii). In addition we have established conditions for accumulation of distinct classes of replicative DNA intermediates (iii).

Finally, we have isolated the particular chromatin (the specific gene with associated proteins), which codes for the ribosomal RNA in Tetrahymena (iiii). This will allow studies of DNA repair on a molecular level of an active gene in the eukaryotic chromosome.

Results of Project No.:	122-74-1-Bio DK
Head of Project:	Dr. Ole Westergaard
Coworkers:	Johnson, B., Leer, J. C., Nielsen, O. F., Piper, P. W.
Title of Project:	The Effect of Radiation on the Discontinous DNA Replication in Eukaryotic Organisms

(i) INDUCTION OF DNA POLYMERASE IN RESPONSE TO RADIATION

Radiation of Tetrahymena with ultraviolet light or electrons causes a considerable increase (up to 50 fold) in the specific activity of a mitochondrial DNA polymerase activity. The activity rises until the cells are able to undergo division and declines thereafter to the normal level of untreated cells. The induction is due to de novo synthesis of enzyme in response to the damage and there is a clear correlation between the dose of radiation (electron irradiation between 10-300 Krad) and the induction of enzyme (see publication list no. 2 for details).

(ii) ACCUMULATION OF REPLICATIVE INTERMEDIATES IN NUCLEAR DNA IN RESPONSE TO DAMAGE OF DNA

Exposure of Tetrahymena to excision repairable damage of DNA (i.e. radiation) results in accumulation of replicative intermediates in the nuclear DNA within the first period of synthesis. The intermediates, which account for up to 90% of the newly synthesized DNA, are believed to represent replicative structures accumulated in front of damage. The system is valuable for studies of the interaction between replication and repair. In particular, it allows studies of repair synthesis at the growing points (for details see publication list no. 1).

(iii) REPAIR OF DNA ON REPLICON LEVEL

Recently we have established growth conditions for Tetrahymena, which makes it possible to prevent joining of newly synthesized replicons (size, around 41S) over longer periods of time (>90 min). This makes it possible to determine the type and the amount of repair, which occurs at the level of replicons. Furthermore, we have been able to show that the chromatin under well defined conditions can be degraded into fragments of replicon size. This strongly suggests that there might be a systematic structural arrangement of nuclease activity or nuclease sensitive sites at

intervals along the DNA. Experiments are now in progress to show if these sites are localized around the initiation and termination sites for the replicons. Finally, these sites might be involved in the crossing over processes (for details see publication list no. 4).

(iiii) STUDIES OF THE PROTEINS INVOLVED IN DNA REPAIR

In Tetrahymena the 500-1000 nucleoli are located peripherally in the nucleus and form ribosomes at a rate per nucleolus which is equal to that of HeLa cell nucleoli. We have recently been able to isolate the ribosomal chromatin (gene plus associated proteins) from the cells and find that the chromatin contains one DNA band only (mol. w. 12.6 x 10^6 dalton) in agarose gels. Furthermore, the ribosomal chromatin sediments as a defined particle with enzymatic activity on neutral sucrose gradients. A number of experiments are now in progress in order to find changes in the enzymatic activities during the repair phase of the gene. It might also by this model system be possible to detect if biological active genes are more sensitive to damage than inactive genes (for details see publication list nos. 3 & 5).

LIST OF PUBLICATIONS

(1) Johnson, B. and Westergaard, O.
Accumulation of replicative DNA intermediates in Tetrahymena after excision repairable damage to DNA. Eur. J. Biochem. in press.

(2) Westergaard, O. and Marcker, K. A.
Accumulation of replicative DNA intermediates in response to damage of DNA in Tetrahymena, published in the book: Radiation and cellular control mechanisms in simple eukaryotic systems (ed. J. Kiefer), Springer Verlag, Berlin, in press.

(3) Piper, P. W., Celis, J., Kaltoft, K., Leer, J. C., Nielsen, O. F. and Westergaard, O.
Tetrahymena ribosomal gene chromatin is digested by micrococcal nuclease at sites, which have the same regular spacing at the DNA as corresponding sites in the bulk nuclear chromatine. Nucleic Acids Res. in press.

(4) Nymann, O. and Westergaard, O.
Accumulation of Replicons in Tetrahymena. Submitted for publication.

(5) Leer, J., C., Celis, J., Kaltoft, K., Nielsen, O. F. and Westergaard, O.
Purification of the ribosomal RNA gene from Tetrahymena in the state of transcriptionally active chromatine. Submitted for publication.

Contractant van de Commissie: State University of Leiden

Nummer van het contract: 102-72-1 BIAN

Hoofd van de researchteams: Prof. Dr. Ir. A. Rörsch

Algemeen onderwerp van het contract: Molecular Mechanisms of the Repair of Radiation Damage

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Summary

Studies on the ploidy of plant cells in relation to radiation sensitivity were continued. Stable cell lines were established from haploid and diploid tabacco plants and from callus tissue. Media were selected that permitted high plating efficiency of isolated Nicotiana protoplasts. The relation between chromosome number and regeneration ability was investigated. The possibility to apply screening techniques for intracellular enzymes by electrophoresis was examined. Two haploid cell lines from plants altered in response to visible light, showed a deficiency in G6PD activity.
After irradiation with a lethal dose of ultraviolet light, crown gall cells are viable for several days, but are permanently inhibited in division. The uptake of DNA by Nicotiana protoplasts was examined. DNA with pyrimidine dimers, was subject to intracellular excision.

Bacterial repair studies were continued at three experimental levels. Complete in vitro repair of UV-induced pyrimidine dimers has been achieved with UV endonuclease, DNA polymerase and DNA ligase. The effect of alterations in the DNA polymerase I enzyme was determined. More detailed studies on the interrelation of repair enzymes and the kinetics of excision repair are in progress. DNA repair enzymes and restriction nucleases are applied in the construction of DNA molecules which contain repair genes and are used in gene amplification. Previously obtained experience in transcription studies of the trp operon is used to investigate the regulation of the uvrB gene.

E. coli bacteria permeabilized by toluene treatment, provide a good system for semi-in-vitro studies. After UV irradiation these cells showed a non-conservative mode of DNA synthesis which is dependent on the uvrA, uvrB and uvrC genes. Mutants of the A and B type could be complemented by the external addition of UV endonuclease. The system is now optimalized for the uptake of proteins.

The influence of mutations in the polA gene in the spontaneous in vivo reversion rate of auxotrophic mutations was measured. More precise chromosomal mapping of several repair genes was continued (dlr, ror, rec and lex). A study on mutagenic post-replication repair phenomena was started by the selection of strains that were non-mutable by ultraviolet light. The results show that, in addition to the known types Rec and Lex, novel Dim-strains were isolated that have differences in radiation sensitivity and recombination ability.

Results of project No.: 1

Head of project: Dr. R.A. Schilperoort/Prof. Dr. Ir. A. Rörsch

Scientific staff: Dr. J.J.M. Dons
Dr. A.M. Ledeboer
Dr. R.F. Heyn (left the laboratory in 1975)
Dr. L. Otten
Dr. E. Wurzer-Figurelli

Title of project: Molecular basis for oncogenesis and differentiation in plants

Progress report:

In order to examine the relation between UV-sensitivity and the ploidy of plant cells a large number of different plant tissue cultures, including haploids as well as diploids, was set up. From most of the callus-tissues suspension cultures were obtained. A variety of liquid media were tested to obtain optimal growth and the conditions for optimal plating-efficiency enabling survival curves to be made, were determined. Reasonable plating efficiencies (about 60%) were obtained using the "nurse tissue" technique where 6000-7000 killed cells are present in the plating medium to provide an as yet undefined growth factor.

Plant cells grown in tissue culture demonstrate chromosomal instability. Since the influence of UV on plant regeneration is investigated the cell lines must be constantly tested for their ability to regenerate whole plants. Using carefully selected media it is now even possible to maintain relatively stable haploid cell lines. The relationship between the ability of the cell lines to regenerate whole plants and the stability of chromosome numbers is determined, using cytochemical techniques for quantifying DNA, developed in this laboratory by Dr. J.J.M. Dons.

Initial experiments showed that cell lines from crown gall tissue are less UV sensitive than normal Straus cell lines. Straus cell lines do not grow either on liquid or solid medium following a UV dose of 35000 erg/mm^2 while they do survive 18000 erg/mm^2. Crown gall cells however do survive a UV dose of 35000 erg/mm^2. In the case of Straus cell lines, the irradiated cells remained stainable with "vital stains" for several days following irradiation demonstrating that the cells were not really dead but rather had lost their ability to divide and form colonies. A search for UV-induced auxotrophic mutants from the haploid cell lines has not been successful. To date no auxotrophic mutants have been isolated.

A screening program was initiated in which 18 enzyme activities could be analyzed in plant cells by cellogel electrophoresis. Almost none of these activities have ever been looked at in plants. In particular, tabacco plants were examined which had maximal growth at a lower than normal light intensity (800 lux) and were obtained from prof. Melchers, Tübingen. Two strains were deficient in glucose-6-phosphate dehydrogenase (G6PD). This is one of the first observations in which a correlation has been found between phenotype and a biochemical lesion. Presently, experiments are done to establish if the enzyme deficiency is the true biochemical basis of the alteration in growth optimum.

With protoplasts (isolated from tabacco leaves) the repair of exogenically UV irradiated E. coli DNA has been studied. Although the experiments show great variability, the preliminary results indicated that the UV-induced thymine dimers present in the E. coli DNA can be removed in the plant protoplasts after uptake. Variability is thought to be due to difficulties in protoplast preparation.

Results of project No: 2.1
Head of project: Dr. P. van de Putte
Scientific staff: Dr. H.L. Heijneker
Dr. C.A. van Sluis
Dr. H. Pannekoek
Title of project: Studies in vitro on the mechanism of repair processes

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Progress report:

Research on the in vitro repair of UV damaged DNA was continued. A new method was developed that allowed both the physical and biological analysis of DNA during excision repair. RF DNA of bacteriophage ØX174 was irradiated and subsequently treated with UV endonuclease, DNA polymerase and polynucleótide ligase. The physical integrity was examined by sucrose gradient centrifugation and biological activity was measured by a transfection assay on repair deficient E. coli spheroplasts. The method was more sensitive and more reproducible than a previously employed technique with transforming DNA and competent B. subtilis.

Exonuclease III is neither essential nor stimulates excision repair, which indicates that the incision break,made by UV endonuclease, is of the 3'OH-5'P type.

The number of newly inserted nucleotides per excised dimer strongly depends on the amount of DNA ligase: this enzyme probably has a high affinity for the complex between DNA and DNA polymerase. The 5'-3'-exonucleolytic function of DNA polymerase I is essential for the repair reaction. Enzyme purified from the mutant polA107, which lacks this activity, leads to a limited amount of strand displacement synthesis without excision of pyrimidine dimers. Complementation could be achieved by addition of "small fragment" of wild type DNA polymerase. Regarding the moderate sensitivity of the PolA107 mutant, we postulated that other cellular exonucleases can replace the missing enzyme function. Preliminary results show that exonuclease V (ATP-nuclease) has complementation activity in vitro. A previously isolated mutant (rorA) was examined in more detail and is specifically affected in repair of X-ray damage. The mutation is located in or very close to the recB gene which together with the recC gene codes for the ATP-dependent nuclease (exo V). This nuclease was purified from the rorA strain and differs in the in vitro activity from the wild type enzyme. The specific activity per unit protein is decreased and the amount of ATP per phosphodiester linkage hydrolyzed is changed.

Progress report, project no 2.1, continued.
The project concerning the in vitro expression of the trp operon has been concluded. The expression of this operon is - in addition to the negative control by repressor - also regulated by a positive control mechanism. Experience obtained by these studies is now applied in the investigation of the regulation of the uvrB operon, which codes for a dimer specific endonuclease. Dissection of a uvrB transducing lambda phage by restriciton enzymes, to isolate a small DNA fragment suitable for transcription studies, is in progress. Our aim is to extend this technique to other repair genes (polA, recA and recBC), to facilitate the purification of the respective gene products. Furthermore it is hoped that a better insight can be obtained in the relation between UV sensitivity and expression of repair genes after UV irradiation.

Results of project 2.2

Head of project : Dr.P.van de Putte

Scientific staff: Dr.B.W.Glickman
Dr.H.Pannekoek
Dr.C.A.van Sluis

Title of project: Identification of genes and enzymes determining radiation sensitivity

Progress report :

The experience on a semi-permeable system was put into practice in the research on the excision-repair in E.coli.This system has the advantage that DNA remains in such a conformation that DNA synthesis can continue, thus resembling the in vivo situation more closely. Compared to in vivo this cell system has the advantage that precursors of DNA synthesis as well as proteins can be introduced from outside. The resynthesis step of excision-repair was used to measure the extent of repair. In cells where semi-conservative DNA replication is inhibited, the UV-stimulated incorporation of deoxyribonucleosidetriphosphates was examined. The non-conservative repair replication was absent in cells unable to carry out an early step in excision-repair, showing that the experimental approach in fact resembles the in vivo repair. At present these experiments are being repeated using density labelling with BUdR.

Semi-permeable cells offer the possibility to complement a defective gene function by external addition of the gene product. The introduction of the UV-endonuclease into uvrA, B or C mutants of E.coli produced results requiring further investigation. It was found that complementation occurred also in uvrC mutant and not only in the uvrA and uvrB mutants. We suggest that in vivo the uvrA and B. gene product (UV-endonuclease) first complexes with uvrC gene product before excision can occur. It is also possible that the uvrC gene product somehow prevents the premature closure of incision breaks by DNA ligase. In vivo experiments showed that the rorA strain repaired single-strand breaks caused by X-rays. The stability of intracellular DNA in the rorA strain in which breaks were introduced using a BUdR-near UV light system, did not differ from the wild type strain. Thus the altered ATP-nuclease has no effect on the repair of this type of damage.

A project on post-replication repair has been initiated. This process, which involves the recombination of newly replicated DNA still containing dimers, is recA dependent. As a test-system the recA-dependent recombinant formation between two ØX174 amber mutants is being used. RF DNA of phage ØX174 is sufficiently stable inside toluene treated cells to be re-extracted and assayed

for biological activity for parental and recombinant DNA.
A new type of radiation sensitive E.coli mutant was isolated (dlr: damaged lambda repair), which is defective in the repair of MMS- or X-irradiated phage lambda and Mu-1. The mutant however does not show itself increased sensitivity to either MMS or X-rays. At present the chromosome localisation of the dlr-gene remains uncertain.
The effect of a number of polymerase I mutations (polA1, polAex1 and resA1) on the spontaneous back-mutation frequency from Arg^- to Arg^+ was studied in isogenic E.coli B strains. In all cases the spontaneous mutation frequency increased by the factor 5-6. Although the polAex1-mutation results in a temperature-sensitive DNA polymerase I, no effect of elevated temperature on the spontaneous mutation frequency was observed.
A study was started on UV-induced mutation in E.coli. A number of mutants which do not give UV-induced mutation-induction were isolated. The genetic locations lead us to believe that several novel mutants have been isolated. These dim strains are also non-mutable by ICR 191 (frame shift mutagen) and by 4NQO and other radiomimetic agents. They still can be mutated by methylating mutagens like nitrosoguanidine. The damage-induced mutagenesis is presently determined more precisely for bacterial damage and infecting phage. Complementation studies between the novel mutants and the known types like recA, lexA and lexB are done to establish if the mutants belong to different operons.

Results of project No.: 3
Head of project: Dr. P. van de Putte
Scientific staff: Dr. P. van de Putte
Dr. G.C. Westmaas
Dr. M. Gassler
Dr. C.A. Wijffelman
Title of project: Integration and excision mechanisms of bacteriophage Mu and its application in genetic engineering.

Progress report:

Bacteriophage Mu is integrated randomly in the chromosome of E. coli and causes mutations by the insertion within operons. During replication, Mu DNA is repeatedly excised and re-integrated in the host chromosome. The investigation of early genes A, B and kil, which are responsible for Mu integration and replication, has been continued (see ZWO, annual report 1975, SON/FABAGEN 11-30-02).

Since damage in bacterial DNA and phage Mu both enhance "illegal" recombination phenomena, and therefore possibly have features in common, recombination of Mu and host DNA is examined. Illegal recombination is highly mutagenic and irrepairable. It is also of interest that Mu integration preferable occurs in the bacterial replication fork, since this structure is believed to be the site where post-replication repair takes place. At present the influence of UV on Mu integration is under investigation.

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R.F. Heyn: "Protoplasts and DNA. Studies towards the genetic modification of plant cell". Ph.D. Thesis. University of Leiden, 1 oktober 1975.

A.J. van der Eb, F.L. Graham, P.J. Abrahams, C. Mulder, H.L. Heijneker, S.O. Warnaar, F.A.J. de Vries and W. Fiers: "Isolatie en Karakterisering van tumorverwekkende genen van dierlijke virussen". Chemisch Weekblad (13 juni 1975), 22-26.

P.H. Pouwels, Hans Pannekoek en C. van Rotterdam: "A transcriptional barrier in the regulatory region of the tryptophane operon of Escherichia coli". J. Molec. Biol. submitted.

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H.L. Heijneker: "Enzymes involved in the repair of DNA, damaged by ultraviolet light". Proefschrift RU Leiden, 15 oktober 1975.

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Contractor : Department of Radiation Genetics and Chemical Mutagenesis

Contract No. : 102-72- 1-BIAN

Head of Research Team : Prof.Dr. F.H. Sobels

General subject of contract : The effects of radiation on genetic and biochemical systems

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Newly developed chromosome types in Drosophila are being used to extend the range of chromosomal rearrangements that can be genetically detected and maintained for analysis in order to gain fresh insights into the mechanisms underlying the formation of induced aberrations.

Several experimental approaches have been developed and are being utilized to test the proposal that most rearrangements induced in mature sperm by radiation may be chromatid-type rearrangements. Thus far, chromatid-type rearrangements appear to be implicated in 1/3 of the cases in which an X or Y chromosome marked at each end has lost one of these markers.

Studies of the maternal repair system that acts on X-ray induced chromosome breaks in mature spermatozoa are being extended to rearrangements produced by a chemical mutagen (diepoxybutane). However, current emphasis is being directed at the problem of whether or not unscheduled DNA synthesis can be demonstrated to occur in meiotic and post meiotic male germ cells of Drosophila similar to that shown by Sega in the mouse in these stages after EMS treatment.

The ascertainment of non-disjunction induction in females is dependent on the spontaneous rates of segregation in males and the viability of the zygotic combinations; both of these factors have been found to be complex and are being investigated.

The chromosome III mutator gene has been demonstrated to increase the frequency of radiation induced X-chromosome loss significantly but not of X-chromosome recessive lethals after treatment of stage 7 oocytes; experiments are being extended to oogonial stages where the mutator gene has been reported to increase the induced recessive lethal frequency.

Research with several mammalian cell systems, which utilize selective genetic techniques to detect "specific locus" mutations and cytological procedures to determine chromosome aberrations, is mainly concerned with

quantitative evaluations and comparisons of radiation and chemically induced mutagenic events in relation to human hazards.

Complications concerning expression time for HGPRT-deficiency mutants obtained by using 6-thioguanine (6-TG) as a selective agent have been sufficiently worked out so that reliable X-ray dose-effect curves in mouse L5178Y lymphoma cells and V-79 Chinese hamster cells can now be obtained. For both systems there is a linear dose response: in the mouse, with an expression time of 11 days, the induced frequency is 1.3×10^{-7} per R; in the Chinese hamster, with an expression time of 7-8 days, the induced frequency is 1.4×10^{-7} per R.

The feasibility of using ouabain resistance and resistance to excess thymidine as additional markers in the mouse and hamster in vitro systems has been investigated. Mutants were induced in both systems with EMS, but at frequencies much lower than 6-TG resistant mutants and too low for our purposes.

A comparison of results obtained for X-ray induced chromosome aberrations after in vitro irradiation of human blood cells with our previously reported data for treated rhesus monkey blood cells indicates: 1) significantly higher frequencies of dicentrics in human lymphocytes, although the "effective chromosome arm number" of both species is almost identical; 2) significantly higher induction of fragments in human cells; 3) symmetrical exchanges (translocations) in human cells, as in the monkey, show a "humped" dose response curve (with a peak at 200 rad), in contrast to dicentrics which increase more than linearly with dose up to 300 rad.

Diploid human skin fibroblasts were used to compare the induction of HGPRT-deficiency mutants and of chromosome aberrations in the same batch of treated cells. Initial results indicate a mutation frequency of about 2×10^{-7} per R and an aberration frequency of 1.2×10^{-3} per R per cell.

Project No. I.1.1

Head of project and scientific staff: Prof. Dr. F.H. Sobels, D. Mendelson

Title of project: The effects of changing the genotype and the physiological environment in females on the repair of chromosome breaks induced by X-irradiation in male germ cell stages of Drosophila.

A. Previous results have shown that: 1) Treatment of females with caffeine affects a genetic repair process that is concerned with the repair of chromosome breaks produced in mature spermatozoa by X-rays. 2) One of the female strains employed carried a chromosome III marked with Ubx e^4 which may have been responsible for a defective repair machinery. Accordingly, two topics are being studied:

1) Localization of the defective maternal-repair factors in Ubx e^4/In (3)Payne, ca gemales. Thus far, it has not been possible to assign the repair factor to a single locus. This may be due to: a) The character of the "marker" used, i.e. the frequency of sex chromosome loss; b) the possibility that we are dealing with a multigenetic system distributed alon the third chromosome. Nevertheless, we have been able to ascertain that the region responsible for repair control is included in the segment demarcated by the markers scarlet (44.0) and ebony (70.7), and therefore includes the Ubx (58.8) locus.
2) Using the cross, ss sbd bx^{34e} females X D Ubx e^4/Ins (3) Payne males, as a test system for rearrangements, transvection rates obtained after irradiation of mature sperm with 3,000R or 2,000R of X-rays are, 6% and 4% respectively. In progress are experiments testing DEB (diepoxybutane) as an inducer of rearrangements. These experiments include sampling of both unstored and stored sperm.

B. After treatment of male mice with EMS (ethyl methanesulphonate), Sega (1974) has shown that there is an unscheduled DNA synthesis in meiotic and post meiotic germ cells. This unscheduled synthesis is taken to be the repair of chemically damaged DNA in these germ cell stages. A similar study has been initiated using Drosophila males in order to detect the repair capability of the different male germ cell stages. Adult Drosophila males are injected with MMS (methyl methanesulphonate) as teh mutagen and H^3 thymidine as the label to detect an unscheduled DNA synthesis. (In further

experiments irradiation will be used as the mutagenic treatment). The testes are dissected, sectioned and covered with autoradiographic emulsion; and the distribution of the label examined. If there is an unscheduled DNA synthesis, then spermatocytes and spermatids will be labeled with H^3 thymidine.

Project No. I.3

Head of Project: Dr. K. Sankaranarayanan

Title of project: How are the marked sensitivity changes from immature (stage 7) to mature (stage 14) oocytes brought about?

The experiments reported in the 1974 EURATOM report focussed attention on the change in sensitivity of female germ cells (stage 7 to stage 14) using an exposure of 3000 R and measuring the induction of dominant lethals and II chromosome recessive lethals in oocyte stages sampled from females ranging in age from about 4 to 36 h at the time of irradiation. These studies have now been extended to a lower exposure of 500 R. The results demonstrate that the pattern of change observed closely parallels that recorded for 3000 R.

For dominant lethals, the oocytes sampled from females of ages ranging from 4 to about 16 h manifest a low level of sensitivity (3-4%); from about 18 h up to 36 h, there is a rather steep rise in dominant lethality (by about 3% for every 1 h increase in age). Beyond this, the increase is more gradual up to 48 h after which there is no further increase.

For the induction of autosomal recessive lethals, although there is a general increase in sensitivity when females of increasing age are irradiated, such an increase is less striking. This in part is due to the low absolute rate of induction and in part due to the fact that the maximum difference between stages 7 and 14 with respect to the induction of recessive lethals is only three-fold.

Further experiments are aimed at measuring the change in sensitivity using yet another end-point of genetic damage namely, the induction of chromatid interchanges.

Project No. I.4
Head of the project: Dr. K. Sankaranarayanan
Title of the project: Exposure fractionation effects in immature oocytes of Drosophila: a re-analysis

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Studies of Traut and Schmidt (1968) on the effects of exposure fractionation on dominant lethal induction in stage 7 oocytes were interpreted as showing a "dose-dependence" of repair in that it decreased with increasing dose. Experiments in progress were designed to demonstrate that the "dose-dependence" of repair is simply another way of describing a curvilinear dose response kinetics of the damage studied.

A series of experiments involving irradiation of stage 7 oocytes with a wide range of exposures delivered either acutely (500 R, 750 R, 1000 R, 1500 R, 2250 R, 3000 R, etc.) or in two or more fractions separated by one hour intervals (2 x 500 R, 2 x 750 R, 2 x 1000 R; 3 x 500 R, 3 x 750 R, 3 x 1000 R; 4 x 500 R, 4 x 750 R) are being carried out to measure the reduction in dominant lethality under the different fractionation conditions. The results available thus far show that (i) the observed dominant lethality with fractionation is closely in line with the one expected on the following premise: if y is the fraction of cells surviving a dose D and y', the fraction surviving the half-dose D/2, then the fraction of surviving cells after two separate half-doses should, on simple probability law be $(y')^2$ provided the effects of the two half doses are independent, the interval of time between the two dose fractions is sufficient for full recovery to take place and there is no change in cell sensitivity within that period of time. This argument can be extended to include doses delivered in n instead of two fractions; and (ii) the observed reduction in dominant lethality after fractionation shows that this decreases as survival decreases after single exposures, one that follows from the argument outlined under (i). Current experiments are aimed at expanding the data already obtained to include other exposures and other fractionation regimes.

Project No. I.5

Head of project: Prof. E. Novitski

Head of project: Nature of action of mutagenic agents (including X-rays) in Drosophila

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New chromosome types in Drosophila are being used to examine the kinds of changes induced in Drosophila by radiation. The major effort has been directed towards the analysis of the behaviour of a newly synthesized large compound autosome. Among the interesting characteristics of this chromosome is a low transmissibility of other structural rearrangements when the two are combined in the same individual. These very low transmissibilities present a new insight into the problems of the segregation and recovery of radiation-induced chromosome rearrangements. One approach in the analysis of the course of aberrant behaviour is to dissect the compound chromosome, and to reconstruct it in various ways, with known (inasfar as possible) contributions of centromeres, centromere regions and centromeric heterochromatin. The first step in this procedure has been accomplished; the long V-shaped compound second chromosome has been broken down by radiation into single armed chromosomes (in six separate instances) and these will now be irradiated and resynthesized to determine, if possible, what conditions determine normal male transmissibility. In addition, this experiment offers a unique opportunity to isolate and identify stable dicentric chromosomes if, as seems a priori likely, the location of two centromeres very close together will cause them to function in concert and therefore improve the transmissibility of the compound.

The use of the compound has made possible new kinds of experiments, of which two are currently underway. Individuals carrying compounds produce gametes either with the compound (two attached homologues) or without it. This leads to a sizable change in the amount of the chromosome material in the two kinds of gametes being produced by that individual. It is interesting to speculate whether such a gross alteration in sperm content may have some influence or the extent of radiation-induced damage. Stocks with standard X-chromosome balancers have been put together to determine whether the frequency of induced sex-linked lethals changes according to the overall DNA content of the sperm.

In another set of experiments, the compounds have been used to isolate small autosomal duplications. This is possible since a female may regularly produced a gamete with two chromosomes (hooked together) and the male contribution may then consist of a small fragment of that same chromosome,

having been produced by radiation-induced breakage. One such duplication for the base of the autosome has been recovered, and, judging from the frequent mosaics it produces, may very well be a small ring chromosome. Other duplications are being generated and will be analyzed in segregation experiments.

During the experiments set up to manufacture these compounds, two cases have been found in which extraneous markers have been attached to the ends of autosomes, without the loss of any essential autosomal material. In addition, one case has been found by B. Leigh (Leiden) and another by J. Puro (Turku). We have examined the latter two cases cytologically; it appears quite conclusive that the duplicating materials has been attached to the end of autosome without any visible loss of material from that autosome, in agreement with the genetic data. These results have a significant bearing on our concepts of the nature of the telomere; the cytological analysis of the two additional cases will be undertaken shortly.

One completed project involves the analysis of the pattern of segregation of non-disjunctional sex chromosomes from X-ray-induced Y-autosome translocation heterozygotes. The results indicate that the X-chromosomes (which are non-cross overs) separate from the translocations (which are cross overs), demonstrating the association of all chromosomes, whether cross overs or not, in a common meiotic configuration.

Project No. II.1
Head of project: Dr. B. Leigh
Title of project: X-ray induced autosomal non-disjunction and associated chromosomal changes in oocytes

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The first experiments this year were carried out with various combinations of compound second chromosomes. In all cases there was a problem with missing classes of exceptional progeny. Therefore it was decided to screen a number of male stocks and determine the rates of compound second chromosome non-disjunction and the viability of exceptional progeny, without using radiation. Females which are heterozygous for a double inversion on the X-chromosome give a high rate of spontaneous non-disjunction of compound autosomes. By mating different kinds of male to a standard type of female it is possible to obtain a relative measure of the amount of non-disjunction in the male. One class of exceptional progeny will receive both second chromosomes from the mother and should have a uniform viability irrespective of which male is used, assuming that this is determined mainly by the compound second chromosomes.

Females of the genetic constitution X,y B/Inscy; j : px were mated to 23 different kinds of males. The rates of non-disjunctional progeny ranged from 15% to 0%. The frequencies of j : px appear to fall into 3 or 4 classes, as though there are distinct rates of non-disjunction in the males. Superimposed on this pattern was another effect, in some crosses equal numbers of exceptions were recovered with maternal or paternal chromosomes while in other crosses there was an apparent deficiency of exceptional progeny with paternal second chromosomes.

Radiation experiments are now being started with males which give a high rate of non-disjunction and equal frequencies of maternal and paternal exceptions.

It is important to get the viability problem eliminated because a full interpretation of the effects of radiation on the non-disjunction of compound autosomes, depends on the relative frequencies of different types of exceptional progeny. In more general terms, the present effort is directed towards eliminating irrelevant experimental variables.

Project No. II.3
Head of project: Dr. B. Leigh
Title of project: The types of chromosomal damage induced by irradiation of spermatocytes

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To sample irradiated spermatocytes, 24 h. old pupae were irradiated and the emergent males mated for three one day broods, with three ♀♀ per per brood. Ring-X and rod-X chromosome males were irradiated with doses ranging from 750R to 200R. Even at the lowest dose, the frequencies of exceptions were higher than those in the control with both types of males.

Some types of exceptions were found with both types of male, for example complete losses and exchanges between the two arms of the Y chromosomes. Other classes of exception were only found when rod-X males were irradiated. For example, chromosomes which resulted from exchange between the X and Y chromosome. Such exchanges could involve either the long arm or the short arm of the chromosome and the break points on the X chromosome were sometimes distal to su(f), which means that they were in the euchromatin.

Attached X-Y chromosomes were recovered from the spermatocytes of irradiated ring-X males. The ring-X chromosome is deficient for the bobbed region and it was found that some of the newly induced X-Y chromosomes have apparently lost the bb region from the Y chromosome. This is in accordance with the hypothesis that such chromosomes result from complex exchanges and loss of one centromere.

Some of the exceptional classes are still being tested.

The types of exception recovered from irradiated spermatocytes make it possible to determine whether a ring chromosome is intact. In one case, unexpected progeny were obtained and a cytological examination confirmed that the ring had opened.

The data so far obtained can be interpreted as indication that irradiation of meiotic stages has generally similar consequences; in males as well as females aberrations originate from chromatid events.

Project No. II.4
Head of Project and scientific staff: Dr. R. Maddern
Title of project: The mechanism of chromosome loss in X-irradiated sperm of Drosophila

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Scattered observations of chromatid rearrangements produced by radiation treatment of mature sperm have been known for a long time. More recently, Leigh and Sobels (1970) and Novitski (1962) have studied the induction of special rearrangements which appeared to require 1) an induction of a chromatid -type of interchange which 2) segregates at the first cleavage division and 3) that the adult organisms carrying the rearrangement is derived from only one of the first two cleavage nuclei. They have raised the question as to whether such cases are not really special, but represent clues to the fundamental mechanism of origin for most rearrangements induced in mature sperm. (Unpublished observations of Brewen indicate that irradiation of mature sperm in the mouse produces predominantly chromatid-type rearrangements).

This proposal is being tested by a detailed examination of partial chromosome losses (fragments). For any fragment to be stable and pass through mitosis it requires the presence of a centromere and telomeres. Two genetic schemes have been devised to study the origin of the telomeres on X-ray induced X and Y chromosome fragments.

In the first scheme (developed by Leigh) the telomeres, as far as possible, are carrying recognizable genetic markers, and from the combinations and number of doses of the markers recovered after irradiation, the nature of the fragment and whether it arose from a chromatid-type rearrangement can be deduced. After treatment with 3000 R thirty four X or Y chromosome fragments, genetically transmissible, were recovered; six could be shown to involve autosomal telomeres, and two could be proven to have resulted from chromatid type rearrangements. The experiment was repeated using an improved scheme with X and Y chromosomes carrying recognizable markers on each end. Of 51 genetically transmissible marked fragments, 16 probably resulted from chromatid-type rearrangements. In addition there were 50 cases of apparent total chromosome losses, (with both tip markers missing), of which 20 proved to carry bb^+ fragments. This approach is limited as telomeres can only be recognized by the presence of a genetic marker whereas they may be beyond the most distal markers available.

The second scheme (proposed by Novitski) overcomes the above limitation of marked telomeres. By using compound autosomes (C(2)L and C(2)R) it is

possible to irradiate gametes carrying ten chromosome ends in diplo 2 sperm, but only six chromosome ends in nullo 2 sperm. The new type of compound chromosome synthesized by Novitski (ref. Project No. I.5) permits these two classes of sperm to be regularly recovered as viable zygotes. By studying the frequencies with which X and Y chromosome fragments are produced after irradiation of the two classes of sperm it will be possible to see if the number of chromosome ends limits the availability of material for capping broken chromosomes. The genetic strains necessary for this approach have been constructed and the experiment will be conducted shortly.

Project No. III

Head of Project : Dr. A.P. Schalet

Title of Project : Quantitative and qualitative characterization of radiation induced heterochromatic rearrangements

In the 1974 report it was noted that because the special Y-chromosome with markers near the end of each arm, $B^{S}su\text{-}f^{+}Y^{L}.Y^{S}y^{+}$, was widely used to score induced partial and complete chromosome "losses", it was of considerable interest to identify the genetic events responsible for the phenotypic changes affecting the tip markers. In particular, the change B^{S}-shape eye -> wild-type eye is usually described as representing a partial chromosome "loss" involving the Y^{L} arm. From experiments described earlier there were approximately 60 fertile males and females which exhibited an X-ray-induced phenotypically complete B^{S} "loss" (43) or mosaically expressed B^{S} "loss" (17).

The results of genetic analysis suggest that less than half of the cases studied arose as simple partial chromosome "losses".

1) There were 10 cases of a B^{S} "loss" in which all offspring carrying a Y chromosome were B^{S}. There were 4 cases of a B^{S} "loss" in which pertinent offspring displayed the exaggerated Hw effect associated with the presence of the Y^{S} tip in double dose and had wild-type eyes. In 1 case a fly mosaic for the Hw exaggeration produced offspring which showed B^{S} only. All 15 cases are interpretable as chromatid-type rearrangements with the 5 cases showing the Hw effect specifically inter-Y arm chromatid exchanges.
2) There were 10 cases in which the "loss" of B^{S} was transmitted and accompanied by a Y-autosome translocation which appeared to involve Y^{L}. These may be considered as 2-break chromatid or 3-break chromosome rearrangements.
3) There were 10 cases in which it was clear that the B^{S} region was not actually lost: 6 involved variegated position-effect rearrangements where the B^{S} phenotype was sensitive to changes in the dosage of heterochromatin; in 4 cases the B^{S} phenotype was partially suppressed by autosomal Minutes, and this suppression, in turn, was sensitive to heterochromatin dosage changes.

The B^{S} "losses" represented by categories 1) and 2) suggest that about 25-40% of the cases examined here could have arisen as chromatid-type changes (see project II.4). The variety of chromosome changes revealed prompts the proposal that it may be profitable to routinely subject to further analysis the F_1 detectable B^{S} "losses" induced by various mutagenic treatments.

Project No. IV

Head of project: Dr. K. Sankaranarayanan

Title of project: The role of mutator genes on radiation-induced mutability in female germ cells of Drosophila

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In the 1974 EURATOM report, results obtained until then on the relative radiosensitivities of the stage 7 oocytes of females homozygous for the mutator gene (μ/μ) and those of females not carrying the mutator gene (+/+) were presented. The genetic damage measured was the induction of X-chromosome losses. These experiments are now complete at exposure levels of 750 R, 1500 R and 3000 R and a few more runs are needed at the highest exposure of 3750 R. The present findings (based on a total count of more than 100,000 progeny) confirm the results reported earlier in showing that the stage 7 oocytes of the μ/μ females are more sensitive to the induction of X-chromosome losses. The induced frequencies, based on pooled results at each of the exposure levels are given below:

	μ/μ	+/+
750 R	0.15%	0.09%
1500 R	0.59%	0.41%
2250 R	1.16%	0.80%
3000 R	1.88%	1.46%
3750 R	3.43%	2.07%

(Note: The frequencies are calculated as the proportion of XO males among the total.)

The stage 7 oocytes of these two kinds of females were also tested for the induction of sex-linked recessive lethals and a total of four experiments at 3000 R have been carried out. In contrast to the results on X-chromosome losses, the recessive lethal studies show no difference between the frequencies observed in the two groups: μ/μ: 4.7% (1318 chromosomes tested) versus +/+: 4.7% (1463 chromosomes tested). Current experiments are directed at exploring the possibility whether any difference is demonstrable in oogonial stages. The choice of this stage was prompted by the contention (Gold and Green, Genetics 1975) that the mutator genes may affect DNA repair or replication and by the finding that after a 100 R exposure, there was a significant increase in recessive lethals in oogonia sampled from the μ/μ females (relative to the +/+ females) manifested by the occurrence of clusters in the former, but not in the latter.

Project No. V.

Head of project an scientific staff: Prof. Dr. F. H. Sobels
Drs. A. W. van der Wielen

Title of project: The effect of several X-ray qualities on the induction of genetic damage in spermatocytes of Drosophila

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Following the recent demonstration by Haendle (1971) that radiation-induced mitotic recombination in Drosophila is dependent on the X-ray spectrum and the radiation intensity, special stocks were designed to test the effect of X-ray quality on the induction of genetic damage in spermatocytes. The male stock was y w^{a} f/B^{S} Yy^{+}; dp b cn bw/+ and the female stock was In(1)sc^{S1L-8R}+d1-49,y; dp b cn bw; e. These stocks make it possible to score losses and partial losses of the sex chromosomes, autosomal crossing-over, and autosomal translocations.

Pilot experiments were carried out, to determine the larval or pupal stage when the most advanced germ cells in the testes, of this male stock, are predominantly spermatocytes. When 0-4 hr old pupae are irradiated with 500R, at 100 kV, the first two one-day broods give relatively high frequencies of exceptions as compared to the following two broods, the successive frequencies are 2.44% (54/2213), 1.7% (44/2645), 0.4% (15/3858), and 0.3% (5/1577). These frequencies are for the pooled data of autosomal crossing-over and partial sex chromosome loss.

It is assumed that chromatid breakage and exchange, either between homologues or heterologues, is being studied by the genetic end points which have been selected. All autosomal crossing-over and a high proportion of the partial sex chromosome losses result from exchanges between homologues.

The stocks are now being adapted to avoid some of the viability problems which were encountered during the first experiments. For example, dp is being replaced by net.

Project No. VI.1

Head of project and scientific staff: Dr. J.W.I.M. Simons
Dr. A.D. Tates
Drs. A.G.A.C. Knaap
Drs. Y.C.E.M. De Ruijter
Dr. A.A. van Zeeland

Title of project: Mutation induction in diploid somatic cells in vitro.

The expression time of radiation induced mutations in mouse L5178Y lymphoma cells is characterized by an optimal expression at day six to seven, followed by a decrease as has been reported earlier. The expression time curve appears to have a plateau from day 11 onwards. The dose response curve after an expression time 11 days does not deviate from linearity and the induced mutant frequency per R is 1.3×10^{-7}.

Because of the peculiar expression time curve in the mouse lymphoma cells the expression time in V-79 Chinese hamster cells was also examined. Cells were trypsinized after the expression period and respreaded in selective medium to avoid effects of cell desity. Optimal expression was found at day seven to eight after irradiation with 600 R and shorter times were found for lower doses. In contrast with mouse lymphoma cells there is no decrease in mutant frequency but a clear plateau. The dose-response relationship is linear and the induced mutant frequency per R is 1.4×10^{-7}.

Preliminary experiments for the selection of mutants from cells taken directly from somatic tissue have been undertaken by trypsinizing one-day old mice and seeding these cells directly for cloning. Only a few transformed clones arise while most of the clones formed in this way are small due to cellular senescence and cannot be isolated for further testing. Therefore, in subsequent experiments rat lung tissue was used. There are indications that enough viable cells can be obtained and that mutants are present.

To be able to select for another marker in the cell lines in use in our laboratory experiments were performed with ouabain and excess thymidine as selective agents. In L5178Y mouse lymphoma cells excess thymidine probably is not useful as the background frequency is high ($\pm$ 1×10^{-4}). The Background frequency of ouabain resistant mutants is very low ($\pm$ 3×10^{-7}) but induction found after two hours treatment with 2.25×10^{-3} M EMS is low also, namely 2.8×10^{-6} and

4.1×10^{-6} in two experiments. In the same experiments thioguanine resistant mutants were induced at rates of 3.6×10^{-5} and 7.3×10^{-5} respectively.

Similar experiments were carried out with V-79 Chinese hamster cells. Background frequencies were low for both excess thymidine and ouabain, but induction of these markers was about ten-fold lower than the induction of thioguanine resistant mutants. Therefore experiments to find a suitable marker will be continued.

To examine the correlation between radiation induced mutations and radiation induced chromosome aberrations in diploid human skin fibroglasts one batch of cells was used in an experiment to measure both kinds of events. The first results suggest an induction of 2×10^{-7} mutations per R and 1.2×10^{-3} chromosome aberrations per R per cell.

Project No. VI.2

Head of project and scientific staff: Dr. J.W.I.M. Simons
Drs. P.C.F.M. Verschure
Dr. A.A. van Zeeland

Title of project: Analysis of 8-azaguanine resistant mutants.

Complementation of HGPRT-deficient mutants can be studied by means of cell hybridization. As mutants we used Lesch-Nyhan cells and mutants selected from human diploid skin fibroblasts and mouse lymphoma cells. The mouse lymphoma cells were selected with 6-thioguanine. The mutants from the fibroblasts were obtained with 8-azaguanine or 6 thioguanine. The HGPRT activity in hybridized cells was determined by enzyme assays and by autoradiography. No evidence for complementation has been found. Experiments were started to study the mechanism of the AG-resistance in mutant tetraploid hamster cells which show no reduction in HGPRT-activity. These mutants were not cross-resistant with 6-thioguanine, but in 6-thioguanine,HGPRT-deficient mutants could be selected from these mutants. The parental line never gave rise to mutants in 6-thioguanine. The electrophoretic mobility of the HGPRT from one induced and one spontaneous mutant was determined and was found to be not different from that of the parental cells.

The HGPRT-activity measured in nmol/mg protein/hour was also measured with labeled 8-azaguanine as substrate. The efficiency of the enzymatic conversion of 8-azaguanine appeared to be 80 per cent that of hypoxanthine for both mutant and wild type, which indicates that the resistance is not a consequence of substrate-specificity. For this characteristic it did not make a difference whether the mutants had been cultured in the presence or absence of 8-azaguanine.

To test whether residual enzyme activity of mutants depends on the concentration of the selective agent, a method is in development to measure in situ the HGPRT-activity in a clone. This method will allow the testing of large numbers of clones immediately after clone formation. In this way it will be possible to test whether the concentration of 8-azaguanine *per se* affects the enzyme level in mutants.

Project No.: VI.3

Head of project and scientific staff: Prof. Dr. F.H. Sobels
Drs. P.P.W. van Buul

Title of project: Comparative studies on the induction of chromosome aberrations in somatic cells and spermatogonia of mouse and monkey.

The experimental work on the dose-response curve for X-ray induced chromosomal aberrations in bone-marrow cells and in spermatogonia of the mouse has been completed, and the statistical analysis of the results is underway.

For interspecies comparison with our results obtained on in vitro irradiated rhesus monkey blood (see report 1974), dose-effect curves for the induction of chromosomal damage after in vitro irradiation of human blood had to be obtained. The radiation doses were the same as those employed in the rhesus monkey experiments, i.e. 100, 200 and 300 rad of X-rays. The recorded classes of chromosome aberrations were dicentrics, reciprocal translocations and fragments.

The results show that:

1. The dicentric frequency at the 300 rad level is significantly higher ($P < 0,01$) in the human lymphocytes than in the rhesus monkey lymphocytes although the "effective chromosome arm number" of both species is almost identical (83 versus 81 for man).
2. With respect to reciprocal translocations we (symmetrical exchanges) obtained further support for our observations after in vitro irradiation of rhesus monkey-peripheral blood lymphocytes (see report 1974) that the dose-response curve for this class of abberations is "humped" with the peak at the 200 rad level. This finding stands in contrast to the frequencies of dicentrics which increase more than linearly with dose, up to 300 rad. These obeservations are of importance, because reciprocal translocations are transmissible stable chromosome aberrations, theoretically expected to be induced by ionizing radiation with the same relative frequency as dicentric chromosomes (Heddle)[1].
3. The induction of chromosome fragments, ranging from the loss of whole chromosome arms down to minute interstitial deletions, is significantly higher in human lymphocytes than in the rhesus monkey at the 300 rad level.

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.To obtain more information about the relationship between symmetrical and asymmetrical aberrations, a project has been instituted in cooperation with Dr. A.D. Tates of this laboratory and Dr. G. Olivieri (Rome, Italy). We are studying Chinese hamster bone marrow cells and cells cultured in vitro to ascertain the ratio between symmetrical and asymmetrical chromatid exchanges obtained after treatment of S-phase and G_2-phase of the cell cycle. The scoring of slides is in progress.

The spermatogonial population is a heterogeneous one. Consequently, the dose-response relationships obtained for induced translocations in spermatogonia of different laboratory animals are difficult to interpret. To get a better insight into this problem a new fractionation experiment was set up in cooperation with Dr. A. Léonard (Mol). Mouse gonia were irradiated using fractionation regimes of 50R+550R with a 24 hour interval between the doses, or 500R+50R with the same time interval. Preparations were made and the scoring of slides is in progress.

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1. Heddle, J.A. Genetics 52 (1965) 1329.

Project No. VI.4
Head of project and scientific staff: Dr. A.D. Tates
Title of project: Are there storage effects for chemically induced genetic damage in mammalian cells in culture?

Normal human foreskin fibroblasts in stationary phase were treated with tetra-ethylene-imino-1,4-benzochinon. Treated cells were stored for 0, 1, 2 or 3 weeks on special storage medium and then analyzed for induced chromosome aberrations. The results showed that there was a decrease in the frequency of chromosome aberrations with increasing storage time rather than an increase. These observations may indicate that 1) a storage effect of the type observed in Drosophila, Neurospora and barley (i.e. an increase of genetic damage with duration of storage) does not occur in human cells in culture or 2) the effect is masked by cell selection and/or repair processes. To inquire whether the absence of a storage effect might have been, at least in part, due to the interaction of repair processes and the effect sought, xeroderma pigmentosum cells in stationary phase were treated with N-acetoxy-AAF. This experiment likewise provided no evidence of a storage effect. The experiments will not be continued at the present time and the available data have been accepted for publication in Mutation Research.

New projects

1. Non-disjunction induction by mutagenic agents in male germ cells of the Northern Vole Microtus oeconomus.

Following the development of a cytological technique for the identification of X and Y chromosomes in spermiogenic stages of Microtus oeconomus (A.D. Tates, P.L. Pearson and J.P.M. Geraedts: J. Reprod. Fert. (1975) 42, 195-198) and following the establishment of a sufficiently large breeding colony of these animals, we have now embarked on a small series of experiments designed to detect the effect of X-rays on non-disjunction. Data cannot yet be given but the scoring of slides is in progress.

2. Correlation between radiation induced mutations and radiation induced chromosome aberrations in diploid human skin fibroblasts

Detailed results will be given in a later report but the first results indicate a correlation of 2×10^{-7} mutations per R and 1.2×10^{-3} chromosome aberrations per R per cell (see also project No. VI 1 Simons et al.)

3. Symmetrical versus asymmetrical chromosome aberrations

In cooperation with Dr. G. Olivieri of the Genetics Institute in Rome and Drs. P.P.W. van Buul in our Department we recently started radiation experiments to obtain more information about the relationship between symmetrical - and asymmetrical aberrations. The aberrations are induced in bone marrow cells from the chinese hamster and also in chinese hamster cells in vitro. The scoring of the slides of these experiments is in progress.

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Laboratory for Physiological Chemistry
Contract No. 102-72- 1 BIAN
Dr. A.J. van der Eb

STUDIES ON THE MECHANISM OF TRANSFORMATION BY ONCOGENIC DNA VIRUSES

There is an increasing believe that viruses may be involved in the induction of cancer in man. Recent evidence suggests that RNA tumorvirus information is present in human leukemic cells and in some other tumors, and it is generally assumed that at least one DNA tumorvirus, the Epstein-Barr virus, is involved in certain types of human cancer. Recently a number of SV_{40}-like viruses have been isolated from man. The finding that these viruses (e.g. BK virus, JC virus) are oncogenic in hamsters, and that they are widely distributed, and persist in a large fraction of human populations, indicates that the possibility has to be considered that they may also be oncogenic in man.

The purpose of the present research project was to study the mechanism of transformation by the oncogenic Adenoviruses and SV_{40}. Considerable evidence has been accumulated in recent years that transformation by these viruses is caused by only one or a few viral genes. The identification and characterization of the transforming genes would, therefore, be of great importance for obtaining an understanding of the process of virus-induced transformation.

We have recently been able to determine the localization of the transforming genes on the DNA of Adenovirus type 5. By using restriction endonucleases, it has been possible to isolate a small DNA fragment which was able to induce transformation. Similarly, a fragment of SV_{40} DNA was isolated, which could induce transformation in vitro. Preliminary experiments have been started to identify the products of these genes, using cell-free protein synthesizing systems.

Project No. : B 1

Research workers: Drs. P.J. Abrahams, Dr. J.H. Lupker, Ir. H. Jochemsen, Dr. A.J. van der Eb
Cooperation with Dr. C. Mulder, University of Massachusetts, Worcester, and Professor W. Fiers, Gent.

Title : ISOLATION OF TRANSFORMING FRAGMENTS OF ADENOVIRUS AND SV_{40} DNA.

Previous experiments had shown that the transforming activity of Adenovirus type 5 was localized within the first 5 or 6% from the left-hand end of the DNA. Attempts were then made to isolate specific fragments with transforming activity. The DNA's of Adeno 2 and 5 were cleaved with bacterial restriction endonucleases, and the fragments were separated and tested for transforming activity. It was thus found that a 7% fragment (molecular weight 1.6×10^6) of Adeno 2 and 5 DNA was capable of inducing transformation in vitro. The transformed cells were shown to contain the Adenovirus specific Tumor antigen, as well as the viral DNA sequences which were used to infect the cells. Similar experiments were carried out with the DNA of SV_{40}. By using the combined action of two restriction endonucleases, it was possible to isolate a 59% fragment of SV_{40} DNA (molecular weight 2×10^6) with transforming activity. The 59% fragment contains the entire early region of the SV_{40} genome (which represents about 50% of the DNA).

In order to identify the proteins which are encoded by the transforming DNA fragment of Adenovirus 5, messenger RNA molecules, isolated from infected cells and selected by hybridization against the transforming 7% fragment, have been translated in cell-free protein synthesizing systems (from wheat germ and ascites cells). Preliminary results indicated that the fragment codes for two proteins.

Further work was concentrated on the characterization of cells transformed by the small DNA fragments, and on attempts to more precisely determine the position and size of the transforming genes.

Laboratory for Physiological Chemistry
Contract No. 102-72- 1 BIAN
Dr. A.J. van der Eb

STUDIES ON THE MECHANISM OF REPAIR OF RADIATION DAMAGE IN MAMMALIAN CELLS.

Studies on the mechanism of repair of radiation damage in mammalian cells are hampered by the complexity of the cell and in particular by the large size and heterogeneity of the cellular DNA.

It has been reported that the repair processes in mammalian cells not only act on their own cellular DNA but also on heterologous DNA, e.g. the DNA of a virus. This type of repair (usually called host-cell reactivation), has been studied with several viruses, e.g. polyoma-, SV_{40}-, herpesvirus. This host-cell reactivation provides the possibility to study the repair processes in mammalian cells with DNA molecules of relatively simple structure.

In this investigation the monkey virus SV_{40} was used, which contains a circular, double stranded DNA molecule as genome with a molecular weight of 3.5×10^6 daltons. The advantage of using this virus is that it is biochemically and genetically well characterized and that it can replicate in human cells. It has been possible, by using UV-irradiated viral DNA, to study the host-cell reactivating capacity of several types of human cells, including various cell lines from patients with defects of the repair of radiation damage.

Project No. : B2
Research workers: Drs. P.J. Abrahams, Dr. J.H. Lupker, Dr. A.J. van der Eb
Title : STUDIES ON THE MECHANISM OF REPAIR OF RADIATION DAMAGE IN MAMMALIAN CELLS

In 1975 the first phase of a study on the host-cell reactivation of ultraviolet-irradiated SV_{40} DNA in normal and radiation sensitive human cells has been finished (Abrahams and van der Eb, Mutation Research, in press). The results obtained in this project can be summarized as follows:

An indirect plaque assay was developed to study the survival of irradiated SV_{40} DNA in human cells. This method has been used to study the host-cell reactivation of UV-irradiated SV_{40} DNA in normal human cells and in cells belonging to the five complementation groups of Xeroderma Pigmentosum. The following percentages of survival of the plaque forming ability of double-stranded SV_{40} DNA were found in XP-cells: Group A 13%; Group B 30%; Group C 18%; Group D 14%; Group E 59%. The survival in a heterozygous XP-strain was almost 100%. The percentage of survival in XP-"variant" cells was 66%. This "variant" cell line was derived from a patient with the clinical symptoms of Xeroderma. However, the cells appeared to be normal with respect of excision-repair, but are probably defective in "post-replication repair". The survival of UV-irradiated double-stranded SV_{40} DNA in XP-"variant" cells was found to be inhibited by 2 mM caffeine, confirming the possibility that this type of cell is defective in post-replication repair. This effect will be investigated in more detail.

The survival of single-stranded SV_{40} DNA in BSC-1 cells was much lower than the survival of double-stranded SV_{40} DNA in XP-cells of complementation group A, which possibly indicates that some repair of UV-damage occurs even in XP-cells of group A. Recently we found that the survival of UV-irradiated double-stranded SV_{40} DNA in a line of XP-cells, belonging to complementation group A, was much higher than was usually found in other cell lines in complementation group A. We found a survival of about 40% in these XP-cells, indicating that variations in the degree of defectiveness also occur within the same complementation group.

Several other cell lines have recently been tested for the ability

to reactivate UV-irradiated SV_{40} DNA: A cell line from a XP-patient which had a normal level of unsceduled DNA synthesis, was found to reactivate UV-irradiated SV_{40} DNA to 75%. This cell line may be similar to be previously described "variant" XP's. Cell lines from porokeratosis and progeria patients were abnormal, in that UV-irradiated SV_{40} DNA was better repaired in these cells than in normal cells (at least at low UV-doses). A cell line from a patient with ataxia telengiectasia (AT), which has been found to be sensitive to X-rays, was normal with respect of UV-repair.

List of publications

1. P.J. Abrahams and A.J. van der Eb

In vitro transformation of rat and mouse cells by DNA from Simian Virus 40.

J. Virol. 16, 206-209 (1975).

2. P.J. Abrahams, C. Mulder, A. van de Voorde, S.O. Warnaar and A.J. van der Eb

Transformation of primary rat kidney cells by fragments of Simian Virus 40 DNA.

J. Virol. 16, 818-823 (1975).

3. P.J. Abrahams and A.J. van der Eb

Host-cell reactivation of ultraviolet-irradiated SV_{40} DNA in five complementation groups of Xeroderma Pigmentosum.

Mutation Research, in press.

4. A.J. van der Eb, C. Mulder, F.L. Graham and F.A.J. de Vries

Transformation of rat cells by fragments of adenovirus DNA's.

J. Virol., submitted for publication.

Contractant de la Commission : Department of Molecular Biology
Université Libre de Bruxelles

N° du contrat : 099-72-1BIAB

Chef des groupes de recherche : J. Brachet

Thème général du contrat : Effets des radiations sur la stabilité de l'information génétique

Project I : Primary effects of radiation on nucleic acids.

a. Computer analysis of ESR spectra of γ irradiated dTMP at 77° K leads to the identification of primary radicals who are progressively converted by step annealing at higher temperatures to secondary radicals and finally into non paramagnetic products, some of which have been identified.

b. NMR studies of protons and deuterium of water during γ irradiation of DNA polynucleotides and polyA + polyU complexes in solution were done at 0° C, -80°C, -196° C in order to determine the role of hydration water at these various temperatures. The variations in protein resonance after irradiation or molecular association are due to an increase or decrease in the proton transfer rate in the hydration layer resulting from changes induced in the macromolecular structure (experiments with D_2O in collaboration with H.J.C. Berendsen, University of Groningen, Netherland).

Project II : Mechanisms of DNA repair in microorganisms and mammalian cells.

a. Microorganisms

- Genetic studies : UV and ionising radiation as well as a majority of chemical mutagens induce an error prone repair mechanism (SOS repair) : after a single UV dose cell survival and mutagenesis increase for 30-40 minutes, then decreases. This increase requires de novo protein synthesis, it is antagonised by cyclic AMP and it could be induced by a blocked replication fork. The roles of the recA and lex gens and the gene for exonuclease I have been further studied.
- Biochemical studies : SOS induction confers the capacity to copy damaged ØX174 DNA. After U.V. it cannot be replicated in vitro by crude cellular extracts or by purified polymerase I, but the replication block is released if the cellular extracts are obtained from SOS induced cells. The DNA replication by these extracts is error-prone, even for non irradiated templates, but more so if the templates(polydT-oligodA ou polydC oligodG) have been irradiated.
- Enzymatic studies : Interactions between E.coli endonuclease I, tRNA and DNA have been studied in E.coli and a DNA condensation protein has been isolated from yeast.

b. Mammalian cells

Heteroduplex DNA molecules probably originating from exchanges between sister DNA molecules have been detected in density gradients or by molecular radioautography; they could eventually participate in a multiplicity reactivation mechanism but not in a major postreplication repair process.
A sensitive immunological procedure for detecting intracellular pyrimidine dimers is under study.

Project III : The establishment and stability of the state of provirus : genetic factors and effects of physical agents.

a. Combined genetic, biochemical and logic analysis has been applied to the control of the decision between production infection and establishment of provirus. One of the results is that transcriptional barriers in phage λ are different in the case of leftwards or rightward transcription. The methods are now being extended to the study of regulation in higher organisms.

b. A "transcription complex" has been isolated from SV40 infected monkey kidney cells. Initiated in vivo, transcription depends on RNA polymerase II and after in vitro incubation, exclusively viral information is transcribed, perhaps in part from supercoiled viral DNA.

c. Any region of a chromosome from E.coli can be transposed on the F factor or other plasmids by illegitimate recombination induced by the "mutator bacteriophage" Mu. This has been applied to the mapping of bacterial genes; it provides a good system for genetic manipulation and for the study of the mechanisms of illegitimate recombination (which is involved in such mutational events as chromosome translocation, and could be involved in oncogenic transformation of mammalian cells).

d. Control of the synthesis of serum proteins has been studied in inter and intraspecific hepatoma-fibroblast cell hybrids : extinction of albumin and α-fetoprotein production has been observed, but secretion of transferrin and of the third component of complement (C3) continues; for this last protein both parental genomes contribute to transcription.

Project IV : Radiation response of somatic cell hybrids and of mouse eggs cultured in vitro.

a. Radiation response of somatic cell inter and intraspecific hybrids
(V. Heilporn, A. Lievens, S. Limbosch, F.Zampetti)
The clones obtained from the fusion of mouse lymphoma cells (L5178YS) and mouse fibroblasts (A9) are much more resistant to X rays than either of the parent strains. In other crosses, radiation resistance, intermediate between parental cell resistance or equal to the most resistant line was found. A cytogenetic analysis of these results has been done and the influence of parental genome on sensitivity has been assessed in some cases.

b. Radiosensitivity of the first differentiation events in the mouse
(H. Alexandre, Y. Gérin)
- Cleavage and blastocyst formation in vitro was studied after irradiation of various cleavage stages and of morulae; the correlation of radiosensitivity with the reduction of ribo to deoxyribonucleotides for DNA synthesis is less well correlated in mice than it was in amphibian embryos.
- In vitro maturation of mouse oocytes after their release from the follicles : maturation in vitro requires pyruvate and a certain level of protein synthesis; effects of γ rays are under study.

Project V : Immunochemical and immunogenetic investigations on the development of of immunocompetent clones in irradiated animals grafted with lymphoid cells

a. Spleens of immunized donor BALBK mice are injected into irradiated or non irradiated BALB/C recipients. The irradiated mice synthesize much more antibodies than the non irradiated ones in which radiosensitive suppressor T cells have not been eliminated.

b. The selection of high affinity receptors during an immune response has been studied by immunofluorescence.

c. Short lined precursors of B lymphocytes appear to be recruited during the secondary response to give rise to lymphoid clones and participate in amplification of the response.

d. The relationships which exist between the products of different activated clone in an immune response have been investigated.

e. Experiments on the mechanisms of tolerance have shown that young B lymphocytes cannot reexpress their surface immunoglobulins following interaction with a ligand.

Résultats du projet n° I.

Chef du projet et collaborateurs scientifiques:
A.J.Bertinchamps; S. Gregoli, R. Mathur-De Vré, M. Olast.

Titre du projet: Primary effects of radiation on nucleic acids.

ESR Investigations. (A.J. Bertinchamps; S. Gregoli, M. Olast)

Following the general pattern of our previous work performed on deoxyadenosine-5'--monophosphate (dAMP), we have used the same computer technique to investigate the mechanism of free radical formation in γ-irradiated frozen solutions of deoxythymidine-5'-monophosphate (dTMP).

Frozen samples of dTMP were irradiated at 77°K and then annealed stepwise to the melting point. During this process, the primary radicals formed at 77°K are progressively converted into secondary radicals and finally into non-paramagnetic products. For dTMP, as well as for most nucleic acid derivatives, no general mechanism governing the post-irradiation events taking place at the free radical stage has been suggested in the literature. Several radical species remain unidentified and no quantitative study on their relative conversion reactions has ever been successfully carried out. The main difficulty in this work is the fact that ESR spectra associated with the radiation-induced radicals are multiplets characterized by g-values which rarely differ by more than 1%. The overall ESR responses therefore consist of composite spectra, formed by different weighted superimpositions of several elementary patterns. Analysis of these composite spectra in terms of their constituents is a challenging problem in ESR research and several attempts to overcome this obstacle have been made, but with poor results.

The method of computer graphical handling of ESR spectra which we have recently developed and described in the Euratom report of 1974, was now applied to the particular problem of the γ-radiolysis of dTMP with fully satisfactory results. Once more this method is proving a powerful tool, of potential widespread applications in radiation research. The temperature-dependent dTMP spectra could be resolved into four distinct patterns: a doublet, T_2, associated with the thymine radical anion ($T^{\bullet -}$); an octet, T_8, associated with the 5-6 dihydro-5-thymyl radical ($\dot{T}H$); (these elementary patterns and their corresponding radical species have already been the object of previous investigations); a quintet, T_5, which we identified as arising from a radical due to OH attachment at C-6 of the thymine ring ($\dot{T}OH$); a quartet, T_4, whose assignement to a cationic radical structure ($T^{+\bullet}$) is, at present, only hypothetical. These four patterns permitted us to

reconstruct with a striking precision, all the ESR spectra arising from the progressive annealing of the γ-irradiated dTMP sample. All these spectra can therefore be represented by the synthetic expression:

$$dTMP\ (\tau) = w_2(\tau)\ T_2 + w_4(\tau)\ T_4 + w_5(\tau)\ T_5 + w_8(\tau)\ T_8$$

In this expression, and with component patterns normalized with regard to their first moment, the four weights $w_i(\tau)$ reveal, at each temperature, the relative concentration of the corresponding radical species. Absolute yields can then be obtained by comparison with a standard. We stress that no other method is actually available to unravel single radical concentration from a composite radical population.

NMR Investigations. (A.J. Bertinchamps; R. Mathur-De Vré)

In the preceding Euratom report it was described that in frozen aqueous solutions of DNA and polynucleotides, hydration water molecules can be distinguished by NMR spectroscopy due to their relatively high mobility compared to bulk water forming a rigid ice-like structure. Several important changes in the hydration characteristics of DNA and polynucleotides resulted when solutions were γ-irradiated at 0°C while no significant changes were observed when irradiations were performed at -19

In an effort to understand clearly the role of hydration water molecules in the radiation damage, we have extended our measurements to study the effects on hydration water proton resonance spectra after: (i) irradiating with different doses at -80°C the solutions of DNA, polynucleotides and polyA + polyU complexes. (ii) sonication of DNA solutions. The characteristics of the spectra (-5°C to -45°C) and E_a values thus obtained after different treatments (non-irradiated; solutions irradiated at 0°C, -80°C and -196°C; irradiating the dry solid before dissolution and sonication of solutions) were all compared and analysed in detail. The three temperatures selected for irradiation of solutions are important because the relat states of hydration and bulk water molecules differ significantly under these conditions. The results obtained after irradiating the solutions at -80°C were of particular importance in showing the direct participation of the hydration water molecules in the overall radiation damage to macromolecules in aqueous media.

The deuteron resonance of D_2O in frozen solutions of DNA (non-irradiated, irradiated at 0°C, -80°C and -196°C), polyA and polyU were recorded at -4°C (in collaboration with Prof. H.J.C. Berendsen, University of Groningen, Netherlands). A comparison of the proton and deuteron resonance results showed that the variations observed in proton resonance spectra after irradiations or molecular association are due to an increase or a decrease in the proton transfer rate in the hydration

layer resulting from structural changes induced in the macromolecular structure.

Some of our exploratory experiments have revealed that it is possible to study the state of intracellular water by applying the method discussed earlier for DNA and polynucleotides. In frozen suspensions of chinese hamster cells in weak salt solutions, temperature dependent proton resonance signal from only a fraction of the total water content (non-freezable fraction) was observed between -5°C and -45°C, with much reduced relaxation times (T_2) values than for pure water and with activation energy values closely similar to those observed for frozen DNA solutions. In the frozen state, proton resonance from the extracellular water (like free water) remains too broad and therefore does not contribute to the observed spectra.

It may be pointed out that several workers have shown by different techniques that water associated with biological macromolecules remains unfrozen well below 0°C. The importance of our work lies in correlating structural changes of macromolecules in solution with the changes in their hydration water and reveal the participation of associated H_2O molecules in the radiation induced damage.

Publications.

S. GREGOLI, M. OLAST and A. BERTINCHAMPS. Free-radical formation in deoxythymidine -5'-monophosphate γ-irradiated in frozen solutions. A computer-assisted analysis of temperature-dependent ESR spectra. Radiation Research (in press).

R. MATHUR-DE VRE, A. BERTINCHAMPS and H.J.C. BERENDSEN. The effects of γ-irradiation on the hydration characteristics of DNA and polynucleotides: I. An NMR study in frozen H_2O and D_2O solutions. Radiation Research (submitted).

S. GREGOLI, M. OLAST and A. BERTINCHAMPS. Spin transfer phenomena in γ-irradiated mixed molecular complexes of DNA nucleotides. I. A computer-assisted ESR analysis of dAMP.dTMP complexes in frozen aqueous solutions. Radiation Research (submitted).

R. MATHUR-DE VRE and A. BERTINCHAMPS. The effects of γ-irradiation on the hydration characteristics of DNA and polynucleotides. II. Proton resonance study in frozen solutions of the mixed solvent $H_2O + D_2O$. Radiation Research (submitted).

Résultats du projet n° II
Chefs du projet et collaborateurs scientifiques : M. Errera, M. Radman, S. Boiteux, P. Caillet-Fauquet, J. Cornelis, M. Defais, D. Kanazir, G.Maenhaut-Michel, J. Rommelaere, G. Villani
Titre du projet : Mechanisms of DNA repair in microorganisms and in mammalian cells.

I. Microorganisms
Genetical and biochemical studies on inducible mutagenic DNA repair (SOS repair)

A) Genetical studies with Escherichia coli and its viruses (P. Caillet-Fauquet, M. Defais, D. Kanazir and M. Radman)
- The mutagenic effect of UV and ionizing radiations and of the majority of chemical mutagens is entirely dependent upon induction of an otherwise repressed error-prone DNA repair system, called SOS repair (1, 2). Some genes involved in this process have been identified.
- The induction kinetics of the SOS repair has been determined : after an optimal mutagenic exposure to UV irradiation, both cellular repair and mutagenic activities are maximal after 30-40 min. incubation in a rich medium, and then decay with a half-life of about 30 min. (M.D., P.C.F., M. Fox and M.R., submitted to Proc. Nat. Acad. Sci. U.S.A.).
- SOS repair and mutagenesis require de novo protein synthesis : chloramphenicol sensitivity of the appearance of the mutagenic activity following a single exposure to UV light, suggests that at least one member of the SOS repair pathway is synthesized de novo only during the first 30-40 min. (manuscript as above).
- UV irradiated phage λ reactivated by the SOS repair recovers full biological activity as deduced from single burst analysis. The growth cycle of irradiated phage is twice as long as that of intact phage (P.C.F. and M.D., submitted to Molec. Gen. Genetic).
- The signal for SOS-induction can be a blocked DNA replication fork, even in the absence of exogenous mutagens. When an E.coli dnaB mutant is grown at 42° C, DNA replication ceases immediately causing the appearance of both induced repair and mutagenic activities (P.C.F. and M.D. in collaboration with N. Henry-Van der Loo).
- Cyclic AMP is an antagonist of SOS repair. Mutants unable to synthesize cAMP (cya⁻) are "superinducible" for SOS repair of their own and phage DNA and are spontaneous mutators. Adding exogenous cAMP inhibits induced repair and mutagenesis (M.D., P.C.F. and M.R., in preparation).
- E.coli cells in the stationary phase of growth are more prone to UV induced mutagenesis then the cells in the exponential growth. Even SOS-deficient mutants (recA⁻ and lex⁻) show some residual mutagenesis in the stationary phase, which is tentatively interpreted as due to the accumulation of leaky gene products, the existence of which has been demonstrated for recA56 mutant (D.K., in preparation).
- The SbcB gene of E.coli codes for exonuclease I, which is involved in genetic recombination. Experiments with isogenic strains in which the sbcB gene has been deleted and then supplied either in cis (parental strain) or in trans (on an episome) suggest that exonuclease I renders DNA repair processes less efficient and more error-prone (D.K., in preparation).

B) Biochemical studies in vivo (P. Caillet-Fauquet, M. Defais, M. Radman)
None of the constitutive E.coli DNA polymerases can copy past pyrimidine dimers in the DNA template. Induction of the SOS repair confers a capacity to copy damaged DNA and this is the probable mechanism of the mutagenic SOS repair (see below). These conclusions were corroborated from

analyses by CsCl equilibrium density gradients, hydroxylapatite chromatography and S1 endonuclease digestion of UV irradiated intracellular phage ØX174 DNA, extracted from intact and from irradiated host cells (P.C.F., M.D. and M.R., in preparation).

C) Biochemical studies in vitro (S. Boiteux, G. Maenhaut-Michel, G. Villani and M. Radman)

a. In vitro reconstitution of the SOS repair (G.V. and M.R.)

Primed single stranded ØX174 DNA has been used as substrate for DNA synthesis by crude E.coli extracts and by purified DNA polymerase I. UV irradiation of the template suppresses DNA synthesis in vitro. Predominant replication-inhibiting lesions are pyrimidine dimers since a treatment with photoreactivation enzyme and visible light releases the replication blockage. Extracts of SOS-induced cells (tif mutants at 42° C, UV irradiation or mitomycin C) show an increased capacity to copy damaged DNA in vitro, thus reproducing the in vivo observations (see section B).

b. An in vitro biochemical assay for mutagenesis (G.V. and M.R.)

The mutagen-induced capacity to copy UV irradiated DNA templates is itself error-prone in an in vitro misincorporation assay, even on intact templates but much more so on irradiated templates. PolydT:oligodA and polydC:oligodG, intact or UV irradiated were used as template-primers and differentially labeled complementary and non complementary deoxyribunucleotide triphosphates were used for incorporation. The frequency of errors in polymerization was estimated from the ratios of respective polymerized radioactivities. This test corresponds to the in vivo genetical observations and appears to be the first in vitro biochemical assay for induced mutagenesis (ref. 3 and G.V. and M.R.).

c. Interactions between E.coli endonuclease I, tRNA and DNA (G.M.M., M.R. and M.D.)

The role of endo (deoxyribo)nucleases whose activity is inhibited and/or controled by tRNA is not known, although this is a major class of endonucleases across the entire evolutionary scale. We have found that purified E.coli endonuclease I-tRNA complex binds to DNA substrates prior to nucleolytic cleavage. Whether particular tRNA species confers a sequence specificity to otherwise nonspecific endo I and which of the tRNA loops is involved in endo I binding, is under investigation.

D) A DNA condensation protein (DCP) from yeast S. cerevisiae (S. Boiteux, G. Maenhaut-Michel and M. Radman)

A DNA-binding protein was partially purified from yeast S.cerevisiae which increases the sedimentation coefficient of the DNA, in neutral sucrose gradients, up to 15-fold. The protein binds to the DNA in a highly cooperative manner and is able to line-up side-by-side and stick together two DNA duplexes. The DCP-DNA complex does not protect DNA against nucleases but the DCP becomes insensitive to proteases, suggesting that, when complexed, the protein is "inside" and the DNA "outside". The DCP-DNA complex has been visualized by electron microscopy. The molecular weight of participating protein(s) is about 45 000 daltons in 4 M urea (to be submitted to Eur. J. Biochem.).

II. Mammalian cells

A. Possible relationship between DNA repair and recombination in somatic Chinese hamster cells (J.Rommelaere)

The search for a possible relationship between DNA repair and recombination in somatic Chinese hamster cells has been pursued, using two experimental schemes which confer on recombinant molecules : either a displaced density detectable by equilibrium centrifugation (10) or an abrupt change in the specific radioactivity along the DNA fibre, revealable by autoradiography (11). The results suggest that the cellular genome

comprises heteroduplex regions which are likely to originate from exchanges between sister DNA molecules. The frequency of these figures is consistent with an uninemic structure of the chromatid; its discrete increase by UV light is incompatible with the involvement of recombinations in the major part of postreplication repair, but does not exclude their participation in a multiplicity reactivation mechanism.

B. Search for a sensitive immunological procedure for detecting pyrimidine dimers (J. Cornelis, J. Rommelaere)

The technique is based on the binding by DNA or chromatin of radiolabelled (I^{125}) antibodies directed against UV irradiated DNA. The specificity of these antibodies is detected by radioimmunoassay and the cellular localisation by radioautography or immunofluorescence. The dose effect curve is linear from 50 to 1000 joules/m^2. Photoreactivation and cellular excision repair decrease considerably the amount of bound antibody (in preparation).

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Résultats du projet n° III
Chef du projet : R. THOMAS and coll.
Titre du projet : L'établissement et la stabilité de l'état de provirus : facteurs génétiques et effets d'agents physiques.

a) Analyse génétique, biochimique et logique du contrôle de l'établissement et du maintien des provirus

(théorie : J. Richelle et R. Thomas ; cellules bactériennes : C. Oambly, M. Gama L. Desmet, A.M. Gathoye, R. Lathe, J.P. Lecocq, F. Salomon et R. Thomas ; cellules de mammifères : P. Gariglio et S. Mousset)

- Etude formelle de réseaux de régulation complexes

Notre méthode d'analyse formelle des réseaux de régulation (Thomas, 1973) s'est déjà révélée utile dans des cas concrets (contrôle de la décision entre infection productive et établissement du provirus chez les bactériophages tempérés : Thomas et Van Ham, 1974). Son emploi est actuellement étendu à d'autres systèmes, et en particulier à la régulation de l'expression des gènes chez les organismes supérieurs (Thomas, Van Ham et Richelle, en préparation). En même temps, les généralisations (introduction du temps comme variable continue, relation modifiée entre fonctions et leur variable de mémoire) apportées au traitement des systèmes séquentiels sont étudiées par des ingénieurs en vue d'applications en dehors de la biologie.

- Mécanisme des contrôles positifs impliqués dans la décision entre lysogénisation et lyse

Le résultat le plus important est sans doute la démonstration claire (Dambly et Lecocq, sous presse) de ce que les barrières de transcription du bactériophage λ sont différentes l'une de l'autre. Une mutation bactérienne affectant la sous-unité β de la RNA polymérase abolit l'exigence de protéine N dans le cas de la transcription "vers la droite", mais pas "vers la gauche".

- Interaction entre cellules de mammifères et virus oncogènes à ONA : la transcription de SV40

Partant des noyaux de cellules permissives (rein de singe) infectées par SV40, P. Gariglio et S. Mousset ont pu isoler une fraction surnageant sarkosyl") contenant un complexe nucléoprotéique dont les propriétés sont celles d'un complexe de transcription. Le surnageant sarkosyl est doué d'une activité endogène de synthèse de RNA. Cette synthèse a été initiée in vivo, car elle n'est pas affectée par la rifamycine AF/013; elle dépend de la RNA polymérase II, car elle est inhibée par l'α-amanitine. Des expériences d'hybridation moléculaire montrent que le RNA synthétisé in vitro dans les surnageants sarkosyl est formé exclusivement de séquences virales, et l'essentiel de la transcription virale a lieu dans cette fraction. Dans le but d'identifier la structure de la matrice de transcription virale, l'effet d'inhibiteurs de la réplication a été étudié; ni l'AraC (inhibiteur de l'élongation des chaînes de DNA) ni la chloroquine

(inhibiteur de l'initiation) n'affectent le taux de transcription observé dans les extraits sarkosyl, pourvu qu'elles soient ajoutées après le début de la réplication virale. Ceci suggère que même la forme superenroulée (I) du DNA viral peut être transcrit par la RNA polymérase II. Jusqu'ici, la transcription précoce n'a pu être détectée dans notre système; ce problème est à l'étude.

b) Recombinaison illégitime : transposition par le bactériophage "mutateur" Mu
(A. Toussaint, M. Faelen, M. Couturier, D. Huismans et F. Van Vliet)

La découverte (Faelen et Toussaint, soumis à J. Mol. Biol.) que le phage Mu peut efficacement transposer des fragments de chromosomes bactériens sur un plasmide a déjà été brièvement mentionnée dans le rapport précédent. Le phénomène est maintenant bien documenté. Des gènes d'apparemment n'importe quelle région du chromosome bactérien peuvent être transposés sur le facteur F ou sur un facteur de résistance, où on le trouve entre deux prophages Mu. Comme on pouvait l'espérer, il s'est avéré possible d'utiliser les fréquences de cotransposition pour localiser les gènes (la méthode est souvent plus pratique que celle qui se base sur les fréquences de cotransduction, car des gènes distants de 4 "minutes" sur le chromosome bactérien peuvent être cotransposés).

c) Contrôle de l'expression génétique dans les cellules de mammifères
(C. et J. Szpirer, A. Résibois, R. Van Geffel et J. Clerx)

C. et J. Szpirer ont poursuivi leur étude du contrôle de la synthèse de protéines sériques dans différents types d'hybrides cellulaires fibroblaste-hépatome. Les quatre protéines sériques étudiées sont sécrétées par l'hépatome, mais pas par le fibroblaste; elles peuvent être considérées comme des fonctions différenciées des cellules d'hépatome.

Dans les hybrides intra- ou interspécifiques dérivés de cellules d'hépatomes de souris, on a observé systématiquement l'extinction de la production d'albumine et d'α-fétoprotéine. Dans des hybrides semblables provenant de cellules d'hépatome de rat, la production d'albumine est bloquée, ou seulement réduite, selon qu'on utilise l'une ou l'autre de deux lignées, apparentées, d'hépatome. Ceci suggère l'existence de différences dans la manière dont certaines lignées d'hépatomes contrôlent leur production d'albumine; le problème est à l'étude.

C. et J. Szpirer on aussi montré que les hybrides d'hépatomes (intra- ou interspécifiques) gardent une capacité limitée de sécréter de la transferrine et une capacité élevée de sécréter le troisième composant du complément (C3). Une analyse plus poussée de la production de C3 dans les hybrides interspécifiques a montré que les deux génomes parentaux contribuent activement à cette production : la sécrétion de C3 par les chromosomes provenant du fibroblaste est donc induite dans les hybrides.

Résultats du projet n° III
Chef du projet : R. THOMAS and coll.
Titre du projet : The establishment and stability of the state of provirus : genetic factors and effects of physical agents.

a) Genetic, biochemical and logic analysis of the control of provirus establishment and maintenance
(theory : J. Richelle and R. Thomas ; bacterial cells : C. Dambly, L. Oesmet, A.M. Gathoye, R. Lathe, J.P. Lecocq, F. Salomon, M.J.Gama and R. Thomas ; mammalian cells : P. Gariglio and S. Mousset)

- Formal study of complex regulatory nets

Our method for formal analysis of regulatory nets (Thomas, 1973) has already proven useful in concrete cases (Thomas and Van Ham, 1974 : control of the decision between productive infection and provirus establishement in temperate bacteriophages). Its use is now being extended to other systems including regulation of gene expression in higher organisms (Thomas, Van Ham and Richelle, in preparation). Simultaneously, the generalizations (introduction of time as a continuous variable, modified relationship between functions and their memorization variable) brought about in the treatment of sequential systems are currently studied by engeneers in view of applications outside biology.

- Mechanism of the positive controls involved in the decision between lysogenization and lysis

The main result is probably the clear demonstration (Dambly and Lecocq, in press) that the transcriptional barreers in bacteriophage λ are different from each other; a bacterial mutation affecting the β subunit of RNA polymerase abolishes the requirement for the N protein in the case of rightward, but not leftwards transcription.

- Interaction between mammalian cells and oncogenic DNA viruses : transcription of SV40

Starting from nuclei of SV40-infected permissive cells (monkey kidney), P. Gariglio and S. Mousset could isolate a fraction ("sarkosyl supernatant") containing a nucleoprotein complex with the properties of a viral transcription complex. The sarkosyl supernatant displays endogenous RNA synthesis (initiated in vivo, as shown by the fact that it is not affected by rifamycin AF/013). This synthesis depends on RNA polymerase II, since it is inhibited by α-amanitin. Molecular hybridization experiments show that the RNA synthesized in vitro in the sarkosyl supernatants carry exclusively viral information, and most of the viral transcription takes place in that fraction. In order to identify the structure of the viral template, replication inhibitors were used; neither AraC (an inhibitor of DNA chains elongation) nor chloroquine (an inhibitor of initiation) affected the rate of transcription in sarkosyl extracts, at least if they were added after viral replication has started. This suggests that

even the supercoiled form (I) of viral DNA molecules can be transcribed by RNA polymerase II. So far, early transcription has not been detected in our system; this problem is under study.

b) Illegitimate recombination : transposition by the "mutator" bacteriophage Mu
(A. Toussaint, M. Faelen, M. Couturier, O. Huismans and F. Van Vliet)

The discovery (Faelen and Toussaint, submitted to J. Mol. Biol.) that phage Mu can efficiently transpose chromosomal fragments on a plasmid was already briefly mentioned in the preceeding report. The phenomenon is now well documented. Genes from apparently any region of the bacterial chromosome can be transposed on the F factor or on a resistance transfer factor, where it is found between two Mu prophages. As expected, it has been found possible to map bacterial genes from their frequencies of cotransposition (a distinct advantage on the use of frequencies of cotransduction is the fact that chromosome lengths up to 4 "minutes" are cotransposable).

In addition to its obvious usefulness as an efficient tool for genetic manipulation and for gene mapping, a major interest of such studies is the insight they should give on the mechanisms of illegitimate recombination. This process is extremely important since it is responsible to a great extent for non-punctual mutations (deletions, duplications, inversions, translocations...); however, its mechanims could hardly be analyzed so far, in view of the low frequency of illegitimate exchanges in other systems.

c) Control of genetic expression in mammalian cells
(C. and J. Szpirer, A. Résibois , R. Van Geffel and J. Clerx)

C. and J. Szpirer have carried on their study on the control of serum proteins synthesis in several types of fibroblast-hepatoma cell hybrids. The four serum proteins which retained their attention are secreted by the hepatoma cells but not by the fibroblasts; these proteins can be considered as differentiated traits of the hepatoma cells.

Extinction of albumin and α-foetoprotein production has been systematically observed in intra- and interspecific hybrids derived from mouse hepatoma cells. Similar hybrids derived from two related clones of rat hepatoma cells either do not produce albumin or produce it at a reduced rate. This suggests the existence of some differences in the way the studied hepatoma clones control albumin production; this problem is now under study. It was also shown that the hepatoma cell hybrids (intra or interspecific) retain the capacity to secrete transferrin at a reduced rate and the third component of complement (C3) at a high rate. Further analysis of C3 production in interspecific hybrids showed that both parental genomes actively contribute to C3 production : induction of C3 secretion is thus observed in these hybrids.

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Résultats du projet n° IV.

Chef du projet et collaborateurs scientifiques : J. Brachet.
Collaborators : H. Alexandre, Y. Gerin, V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti.

Titre du projet:
1. Radiosensitivity of the first differentiation events in the mouse.
2. Radiation response of somatic cell hybrids.

1. a. Cleavage and blastocyst formation in vitro (H. Alexandre, Y. Gerin).

- We showed previously that low doses of X-rays inhibit the in vitro hatching of mouse blastocysts. Since this could be explained by a smaller number of cells, we performed cell counts for control and irradiated embryos after about 90 hrs of culture. A very high heterogeneity was observed in the distributions, showing great asynchrony in cleavage. A dose-proportional increase of the left part of the diagrams, corresponding to embryos of less than 20 cells, is obvious after irradiation of 2-cell stages. This shows that the 50% inhibition of cavitation induced by 200 R and the total inhibition after 500 R correspond in fact to very early killing. Using the same technique, it has been shown that irradiation of morulae induces a dose-proportional delay in cleavage, with an almost total blockage after 500 R; but, in that case, cavitation still occurs. We are now selecting more precisely the stage of cleavage and the X-ray doses needed in order to obtain a high percentage of trophoblastic vesicles. These conditions seem to be, for the time being, the following : 8-cell stage and doses ranging between 300-400 R.

- We performed further experiments for studying the regulation of DNA synthesis during cleavage.

It is now certain that a small proportion of uridine is reduced and incorporated in DNA as thymidine or deoxycytidine : CsCl gradients showed that radioactivity associated with carrier DNA after running extracts of blastocysts incubated with ^{3}H uridine disappears after DNAase treatment, but does not disappear after thermic or alcaline denaturation.

An exhaustive autoradiographic study has shown that the labelling index of RNAase treated preparations is unaffected in 4-8 cell stages when X-irradiation was applied at the 2-cell stage; a moderate effect has been observed at the morula stage (control : 90%, 200 R: 70%, 500 R: 44% labelled nuclei), which is presumably a consequence of the death of some of the blastomeres. A very similar conclusion could be drawn for irradiated morulae, where 500 R exerts an immediate arrest of development with drastic cellular damage, but a decrease of about 30% only of the labelling index.

In conclusion, in contrast with the amphibian situation, radiosensitivity of cleaving mammalian eggs is not strictly correlated with a high sensitivity of the reduction of ribonucleotides to deoxyribonucleotides. This suggests an interesting difference between Amphibians and Mammals in the biochemical pattern for the biosynthesis of the regulatory enzymes involved in this pathway.

b. In vitro meiotic maturation of mouse oocytes (H. Alexandre, Y. Gerin).

- In order to study the radiosensitivity of that very important step in development, we undertook an analysis of the mechanisms of in vitro maturation in mouse oocytes after their release from the follicles. We were able to confirm the lack of any sign of maturation in the absence of pyruvate; chromatin condensation (perhaps corresponding to diakinesis) still occurs in the absence of Ca^{++}. Chromatin condensation is the only event which happens in the presence of parachloro-mercuribenzenesulfonate

(PCMBS), an agent known to induce complete maturation in amphibian oocytes in the absence of any steroid (Brachet et al. 1975). Experiments with cycloheximide have shown that a certain level of protein synthesis during the first hours after release from the follicles is required for the steps of maturation following chromatin condensation.

2. Radiation response of somatic cell hybrids (V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti).

The study of the X-ray response of somatic cell hybrids has been extended this year to different intra- and interspecific hybrids. This work was performed on hybrids resulting from three crosses : the first between mouse cells from different established cell lines (lymphoma cells and fibroblasts) and the two others between Chinese hamster fibroblasts and either mouse fibroblasts or mouse lymphoma cells. From each cross, several clones were isolated and for each clone the karyotype and the survival parameters after X-irradiation were determined.

The results obtained, summarized in the accompanying table, show that :

- Hybrid clones derived from the cross between mouse lymphoma cells (L5178YS) and mouse fibroblasts (A9) are much more resistant to irradiation than either of the parental strains.

Parental cell lines although both derived from mouse, do not have the same chromosome complement. Thus, in addition to a possible ploidy effect, the higher resistance of these hybrids could be result from some interaction between the two genomes.

Lymphoma cells contain only telocentric chromosomes, while about one third of the A9 chromosomes are biarmed. Therefore, in the cross L5178YS x A9, these biarmed chromosomes serve as A9 markers. The chromosome loss is limited (+ 10-15%) and random.

- Hybrid clones derived from the cross between mouse fibroblasts (A9) and Chinese hamster cells (a23) have survival parameter values which are intermediate between those of parental lines. The loss of chromosomes from these interspecific hybrids is small (+ 10-15%).
- Finally, hybrid clones obtained by fusing Chinese hamster cells (a23) and mouse lymphoma cells (L5178YS) exhibit a resistance similar (for clones α, γ, δ) to that of the more resistant (a23) or even higher (for clone β). A massive loss of chromosomes (60-65%), which are essentially of mouse origin, is observed in clones β, γ, δ. This fact could explain why hybrid clones γ, δ display the same X-ray characteristics as those of Chinese hamster cells. The higher degree of resistance to radiation damage observed for hybrid clone β remains, however, unexplained. Hybrid clone α has the same survival properties as hybrid clones δ and γ while having lost much fewer chromosomes (10%). It is possible that this hybrid, which has a higher number of hamster biarmed chromosomes than expected, has lost as many mouse chromosomes as the three other clones, but contains two sets of hamster chromosomes. Further chromosomal analysis of the hybrid cells, by banding procedures, is required before drawing any conclusion on the relationship between survival to X-rays and chromosomal constitution.

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H. Alexandre & N. Herremans (1975) Incorporation de l'uridine ^{3}H dans le DNA des oeufs de souris en segmentation. Arch. int. Physiol. Bioch. 83, 165-166.

H. Alexandre has participated to the organization of the EMBO Course on Differentiation. Rhode St. Genèse, October 1975.
- He spent 3 months at the Department of Zoology, University of Oxford (June, July, August 1975).
- He attended the Contact Group Meeting on the Hereditary effects of Radiation in London (November 19-21, 1975).

A. Lievens has presented a communication entitled "Survival of synkaryons and of the two parental strains following X-ray irradiation" at the Meeting on Cell Tissue and Organ Culture. Radiobiological Institute TNO, Rijswijk (May 1975).

V. Heilporn and S. Limbosch have participated in the Poster Demonstration at the Second International Conference on "Differentiation" Copenhagen (September 1975).

F. Zampetti has performed research work at the Paterson Laboratories of the Christie Hospital and Holt Radium Institute, Manchester (from Oct. 1st to Nov. 15th, 1975).

Karyotypes and survival parameters of parental and hybrid cells.

Cell Type	Mean N_o of chromosomes (range)			Survival parameters	
	Total	Biarmed [x]	Telocentric	Do	n
a23 (TK^-) Chinese hamster cells	23 (21-26)	13 (11-15)	10 (7-12)	160	2.2
A9 ($HGPRT^-$) Mouse L cells	53 (50-57)	19 (17-25)	34 (28-37)	90	2.4
L5178YS X-ray sensitive mouse lymphoma cells.	37 (31-41)	0	37 (31-41)	40	2.0
Intraspecific hybrid:					
L5178YS x A9	expected: 90	19	71		
clone 1	83 (77-90)	18 (14-24)	65 (56-74)	170	1.6
clone 2	76 (66-86)	16 (12-21)	60 (45-71)	140	1.8
clone 3	81 (69-91)	14 (11-19)	67 (56-75)	155	4.0
Interspecific hybrids:					
A9 x a23	expected: 76	32	44		
clone a	68 (59-77)	31 (27-36)	37 (31-43)	125	4.0
clone f	66 (61-74)	28 (20-34)	38 (33-44)	110	3.2
clone h	64 (58-72)	25 (22-28)	39 (31-50)	135	2.4
L5178YS x a23	expected: 60	13	47		
clone α	55 (47-66)	21 (16-28)	34 (25-42)	185	1.6
clone β	24 (20-29)	15 (12-20)	9 (4-11)	205	1.4
clone γ	23 (19-27)	15 (13-19)	8 (4-10)	160	1.6
clone δ	27 (21-30)	15 (12-17)	12 (11-15)	165	1.6

[x] Biarmed : meta + submetacentric chromosomes.

Résultats du projet n° V

Title : Immunochemical and immunogenetic investigations on the development of immunocompetent clones in irradiated animals grafted with lymphoïd cells.

Investigators : J. Urbain, R. Jeener, G. Urbain-Vansanten, A. Van Acker, C. De Vos-Cloetens, V. Hooghe, B. Mariamé, C. Bruyns, N. Tasiaux, R. Leeuwenkroon.

Irradiated animals whose immune function is impaired can recover immunocompetence after a lymphoïd graft. The degree of protection which is given depends on the quality of antibody produced and on the acceptance of the graft. Therefore all studies increasing our knowledge about the mechanism of development of clones (selection of high affinity antibody) and about the mechanism of establishment of tolerance should be useful in this regard.

1) Mice from an inbred strain have all the same genetic repertoire. Skin grafts or kidney grafts are easily accepted by members of the same strain. The results are quite different for lymphocytes. This has been demonstrated in the following way. Donor mice (BALB/c) which have been immunized against TMV or hemocyanine were killed and their spleen cells transfered both in non irradiated or irradiated BALB/c mice. The results are quite different for the two types of transfer. Irradiated mice, repopulated with syngeneic lymphocytes synthesize much greater amounts of antibody than syngeneic non irradiated mice. Apparently some feedback mechanism appears in non irradiated recipients and this feedback mechanism (suppressor T cells) is very radiosensitive. Experiments are now performed to understand this feedback mechanism.

2) It is widely assumed that the gradual increase in binding affinity during an immune response is due to selection of cells bearing high-affinity receptors as the antigen concentration decreases. However we have previously shown that the increase is followed by a decrease. This decrease does not impair high-affinity memory cells since upon boosting high-affinity antibody is immediately synthesized. Using membrane immunofluorescence, we obtained results showing that as the binding affinity decreases, cells bearing receptors recognizing the idiotypic specificities of high affinity antibody appear. The appearance of autoantiidiotypic antibody would represent a normal feedback mechanism of any immune response.

3) Using combined immunofluorescence and autoradiography we have studied the mechanism of development of lymphoïd clones. During the secondary response, many plasma cells which appear are not the clonal descendants of these cells which have been activated by antigen during the primary response. Many plasma cells are recruited from short lived precursors of B lymphocytes. This would easily explain the fact that most plasma cells appearing during a secondary response can be labelled even if tritiated thymidine is given before antigen injection.

Therefore amplification during an immune response is not only due to clonal proliferation but also to recruitment.

4) It was previously shown that heterogeneous specific immunoglobumins which are synthesized in response to antigen injection are not just a random collection of immunoglobulins which happen to fit but that some definite relationship must exist between them.
Indeed there is a sharing of idiotypic specificities between different components of anti-TMV antibodies synthesized by an hyperimmunized rabbit. It seems therefore possible that idiotypes are involved in regulatory phenomena and more especially in the phenomenon of recruitment.

5) Despite the fact that immunological maturity is only attained two weeks after birth in mice, cells bearing receptors for a variety of antigen can be detected. Precursors of plasma cells are thus present but cannot function at this stage. After induction of capping and endocytosis with antiimmunoglobulin sera, no resynthesis was observed for young B lymphocytes.(B lymphocytes taken from foetus or from newborn mice up to one week old). This is in strong contrast with the case of adult lymphocytes which showed a full reexpression of immunoglobulin receptors when similarly treated.
Moreover intrafoetal injection of hemocyanine (Hcy) led to a strong decrease in the number of Hcy binding cells in these foetus. Similar treatment of adult lymphocytes did not led to any decrease in the number of antigen binding cells. We can therefore conclude that young and adult B lymphocytes have a different physiological behaviour upon interaction between surface immunoglobulins and a ligand. The non reexpression of immunoglobulin receptors in young lymphocytes could be the first step of tolerance induction in neonates or in adult bone marrow.

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The University of Dublin, Trinity College
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Professor F.G.A. Winder

STUDIES ON THE MECHANISM OF ACTION OF ENZYMES OF DNA REPAIR AND ON THEIR INDUCIBILITY

Further studies have been made on the properties of the three types of complex between DNA and the ATP-dependent deoxyribonuclease from Mycobacterium smegmatis which were defined in the previous report, and we are trying to understand their roles in the overall action of the enzyme.

Complex A appears to consist of molecules of enzyme bound to the ends of molecules of DNA and engaged in their digestion. Complex B appears to consist of enzyme bound to other sites in DNA, which appear to be limited in number. Formation of this complex probably reduces the availability of enzyme for formation of complex A and, hence, for digestion of DNA; though it does not appear to reduce the availability of DNA for digestion. That enzyme and DNA can interact in the absence of ATP, at least under some circumstances, can be inferred from the kinetics of digestion subsequent to addition of ATP; however, the interaction is so loose that no ATP-independent complex formation can be demonstrated more directly.

It has been shown that all agents which, in M. smegmatis, induce increased synthesis of the ATP-dependent deoxyribonuclease and of a DNA polymerase, produce strand breaks or alkali-labile regions, while other inhibitors of DNA synthesis which do not induce synthesis of these enzymes do not affect the DNA in this fashion. It appears that the damage to DNA by the first class of agents is involved in induction of the enzyme synthesis. Attempts are being made to show analogous effects in Escherichia coli.

We have proceeded further with the investigation of the deoxyribonucleases of Aspergillus nidulans in order to investigate their possible roles in radiation repair and recombination. Five deoxyribonucleases have been partially purified and characterized. Their amounts generally vary markedly with the nutritional status of the organism. Their activities in mutants with increased sensitivity to ultraviolet light and altered levels of recombination are under investigations. We are also endeavouring to obtain mutants lacking one of the deoxyribonucleases.

Project No. : 1

Research workers: Mr. T.F. Creedon, Dr. G.R. Campbell, Professor F.G.A. Winder

Title : Mechanism of action of a nuclease involved in radiation repair in bacteria

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Further attempts have been made to understand the action of the ATP-dependent deoxyribonuclease from Mycobacterium smegmatis on linear double-stranded DNA, and the roles in this action of the three types of enzyme-DNA complex which were defined for this enzyme in the previous report.

Complex A is the complex in which the DNA is broken down processively to yield almost entirely acid-soluble fragments. With DNA of m.w. 16×10^6 the breakdown takes about 60 sec. Complex A does not appear to dissociate to a significant extent before digestion is completed: (a) ultracentrifugation, following addition of a large excess of unlabelled DNA during the reaction and further incubation, did not reveal any evidence that DNA of intermediate size was released by the enzyme; (b) the time for disappearance of the complex agrees with the minimal time for digestion of all DNA in a reaction mixture with a large excess of enzyme.

Even in the presence of excess enzyme, only a small proportion of DNA is normally bound in complex A, and formation of complex A can go on continuously during the reaction under these circumstances, though the rate of formation is maximal during the first few seconds at 37°. The slow rate of complex formation is not normally determined by a low second order rate constant of reaction of enzyme and DNA to form this complex, as can be seen from the effects of increasing the concentration of enzyme and DNA at constant ratio. The slowness of complex A formation under excess enzyme conditions may be due in part to binding of enzyme in complex B, but slow formation can be observed under conditions when Millipore binding studies show a substantial proportion of apparently free enzyme, though this enzyme may be in a loose complex similar to the ATP-independent complex C. It is possible that some activation of the enzyme or modification of the DNA has to occur for complex A formation: when linear lambda DNA was used as substrate at 15° there was an extended lag period before complex A formation or product release started but when the enzyme and DNA were preincubated together without ATP this lag period was eliminated, presumably by removal of single-strand ends; also when enzyme was allowed to act on two differently labelled DNAs, preincubation of the enzyme with one of these

in the absence of ATP resulted subsequently in preferential digestion of that DNA for several minutes, even though no stable complex formation occurred until ATP was added.

In spite of the fact that complex A normally increases in amount during the first 60 sec of reaction at 37°, there is usually a linear release of product during this period. This seems to suggest that the average speed of the enzyme declines during the course of its action on a molecule of DNA.

Complex B is defined as the fraction of Millipore-bindable complex in which the DNA is not digested processively by the endogenous enzyme. This is formed more rapidly than complex A: at 15°, under conditions where complex A formation continued through the whole 10 min reaction period, complex B formation was complete in 30 sec. About 5 times as much complex B as complex A was found when enzyme was in excess, and 15 times as much when DNA was in excess. The effect of varying ATP concentration on complex B formation is similar to that on complex A formation.

Complex B may contain two components, one with a half life of about 60 sec and the other with a much longer life. Breakdown of both components must be by dissociation, since it yields neither complex A nor products. When enzyme is in excess, all of the DNA is either in complex B or complex A: hence, in view of the fact that degradation of the DNA can be faster than dissociation of complex B, this must indicate that free enzyme can attack complex B, presumably converting it to complex A. Enzyme forms complex B rapidly with circular lambda DNA, although complex A does not form until the molecule is linearized.

Complex B probably consists of enzyme bound to DNA at a limited number of sites, perhaps nicks or easily denatured regions. Since substantial amounts of free enzyme can co-exist with complex B, in spite of its rapid formation and slow dissociation, it does not appear that enzyme can bind to many sites in DNA. Further, the dissociation curves do not suggest that several dissociation events are required to free the DNA from complex B.

Project No : 2

Research workers: Dr. G.R. Campbell, Mr. A.W. MacNaughton, Mr. S. Brady, Miss A. Conneely, Professor F.G.A. Winder

Title : Induction of enzymes related to DNA repair

It was reported previously that a number of treatments, including ultraviolet irradiation, methyl methanesulphonate, ethyl methanesulphonate, nitrogen mustard, mitomycin C, hydroxyurea and iron limitation, induce increases in specific activities of a DNA polymerase and of an ATP-dependent deoxyribonuclease in Mycobacterium smegmatis. Evidence was obtained, and has since been extended, that increased synthesis of the enzymes is involved. A number of other treatments, including nalidixic acid, 5-fluorouracil, ethidium bromide, acridine orange and caffeine, under conditions where they inhibit DNA synthesis to a similar extent to those in the first class, do not induce increases in specific activities of these enzymes. It is postulated that increased synthesis of the enzymes is induced by DNA damage produced by agents in the first class.

A method of fairly rapid lysis of this bacterium has been developed. Using this, we have found that treatment of the bacteria with the agents in the first class leads to marked reduction in the molecular weight of their DNA in alkaline sucrose gradients, indicating the formation of single-strand breaks or alkali-labile regions. Treatment of the bacteria with agents in the second class has no effect on the single-strand molecule weight of their DNA. Thus, the results support the above hypothesis.

We have found that enzyme induction by the agents takes place under several growth conditions. On the other hand, variation in growth conditions, such as changes in growth medium, temperature, oxygen tension, visible light, or the presence of radical scavenging agents, do not themselves alter the 'normal' levels of the enzymes. Hence, a level of DNA damage beyond what might occur under the normal range of environmental conditions appears to be necessary for the induction of the enzymes.

Attempts have been made to find similar phenomena in Escherichia coli. The results have been rendered uncertain by the difficulty in assaying DNA polymerases and deoxyribonucleases accurately in extracts

of this organism. Improved methods of assaying these enzymes without extensive purification are under investigation.

Characterization of the deoxyribonucleases of Aspergillus nidulans has continued, in view of the availability of mutants with increased sensitivity to ultraviolet light and altered levels of recombination. Five deoxyribonucleases have been partially purified and characterized.

Two of these deoxyribonucleases (DNAase 1b and DNAase 4) are present in all cultures examined, while nitrogen-limited and carbon-limited cultures possess different acid deoxyribonucleases (DNAase 2 and DNAase 3, respectively). DNAase 4 activity in carbon-limited cultures is considerably reduced by an endogenous inhibitor. Another deoxyribonuclease (DNAase 1a) is present only in nitrogen-limited cultures. A. nidulans does not seem to possess the S_1 nuclease present in Aspergillus oryzae, while A. oryzae crude diastase seems to lack four of the above deoxyribonucleases but has large amounts of DNAase 4. Further purification and characterization of DNAase 1a and DNAase 4 is in progress.

We have commenced an investigation of the ultraviolet-sensitive mutant strains from G.J.O. Jansen, and preliminary results suggest that one mutant may have an altered form of DNAase 2. An attempt is also being made to obtain mutants with altered sensitivity to ultraviolet radiation by selecting for strains with low deoxyribonuclease activity.

Publications

1. A.H. Johnson, T. Creedon and F.G. Winder, Complexes between deoxyribonucleic acid and the adenosine triphosphate-dependent deoxyribonuclease from Mycobacterium smegmatis. Biochem. Soc. Trans. 2 1334-1336 1974.

2. G.R. Campbell and F.G. Winder, Deoxyribonucleases and a deoxyribonuclease inhibitor of Aspergillus nidulans. Proc. Soc. Gen. Microbiol. 2 87 1975.

Contractor:	University College, Galway, Ireland.
Contract No:	127-74-1 BIO EIR
Head of research team(s):	Dr. James A. Houghton
General subject of contract:	The effects of radiation on the blue-green algae.

The blue-green algae are more resistant than bacteria to UV irradiation and they also show extremely efficient photoreactivation. It has been suggested that they may have two photorecovery processes, one for the photoreactivation of pyrimidine dimers, the other for the repair of photosynthetic damage. The presence of a dark repair system has also been proposed.

In this contract, the effects of radiation, particularly UV, on the unicellular blue-green algae Gloeocapsa alpicola and Synechocystis pevalekii are being investigated. Their mechanisms for radiation protection and repair are being studied and also the mutagenic effects of radiation and its effects on genetic exchange.

During the last year three lines of approach to the contract have been taken:

(1) A detailed study of the effects of UV on cell survival in Gloeocapsa alpicola. In particular, a comparison of the effects of far (λ 254 nm) and near (λ 300-350 nm) UV have indicated that near UV has a much greater inhibitory effect on photosynthesis than far UV and the carotenoid pigments may play an important role in protecting the photosynthetic system from the effects of near UV. The influence of the growth cycle on UV survival and the photoreactivation system have been investigated and the presence of a caffeine sensitive dark repair system demonstrated.

(2) A detailed study of mutagenesis in Gloeocapsa alpicola has been undertaken and baseline data on mutagenic induction has been acquired using a variety of mutagenic chemicals for comparison with the mutagenic effects of radiation. Whilst a variety of mutants have been isolated using chemical mutagens, UV has proved virtually non-mutagenic for the phenotypes tested and this is being investigated further using UV and other radiations.

(3) In order to study the effects of radiation on genetic exchange in blue-green algae it has been necessary to demonstrate transformation and transduction in Gloeocapsa alpicola. We have succeeded in reporting the first examples of these phenomena in Gloeocapsa and also the first demonstration of intergeneric transformation in blue-green algae. We are now in a position to continue the study and examine the effects of radiation on these transfer processes.

Results of Project No.: 1

Head of Project and Scientific Staff: Dr. James A. Houghton, Professor L.K. Dunican, Dr. C.E. Buckley, Imelda Devilly, Evelyn Corcoran.

Title of Project: The effects of radiation on Gloeocapsa alpicola.

UV Survival

UV survival of G. alpicola was found to differ markedly depending on whether a far (λ254 nm) or near (λ300-350 nm) UV source was used for cell irradiation, near UV having a much greater inhibiting effect on cell survival. Photo-reactivation of near UV irradiated cells was also much less efficient. Caroteno pigments may play an important role in protecting the cells against near UV damag reduction in carotenoid levels reduced the near UV survival rate. Irradiation of G. alpicola cells by near UV caused a significant reduction in the carotenoid levels. Far UV had no such effect.

The photosynthetic rate of G. alpicola dropped very sharply after the cells were exposed to UV irradiation. Near UV had a greater inhibiting 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 quick recovery after exposure to far UV could suggest that inhibition was merely due to the irradiating wavelength being unsuitable for photosynthesis, whereas near UV may have a much more pronounced effect on the photosynthetic pigment system and carptenoids may play a part in protecting the cell against this damage. Work is being continued on the effects of UV on cell survival and on photoreactivation A caffeine sensitive dark repair system has been demonstrated.

Mutagenesis

The mutagenic effects of UV on the cells of G. alpicola have been compared w a number of mutagenic chemicals. NTG, EMS and MMS were all found to induce a hi frequency of antibiotic resistant mutants, the frequency depending on the mutagen concentration and exposure time. However, in spite of using a wide range of UV doses from near and far UV sources, mutant isolation has been largely unsuccessfu The only mutants that have been obtained, at low frequency, have been those exhibiting increased resistance to polymyxin-B and a number of minute colony formers have also been isolated. However, UV appears to be virtually non-mutage

Radiation mutagenesis in G. alpicola is being investigated further.

Genetic Transformation

Prior to studying the effects of radiation on G. alpicola transformation, an efficient means of isolating good yields of biologically active blue-green algal DNA was devised and yields of 1.5 mg DNA/g wet weight cells with no contaminating protein were obtained. A transformation procedure was designed and using this method the transfer of the streptomycin resistance marker from Str^r G. alpicola to the wild-type strain was successfully demonstrated with a frequency of 3.1×10^{-3}. More significantly, intergeneric transformation of the streptomycin resistance marker from G. alpicola to A. nidulans has also been demonstrated. Using Donor DNA from Str^r G. alpicola, streptomycin resistant A. nidulans transformants were isolated at maximum frequency of 2.6×10^{-3}, that is 6×10^3-fold higher than the frequency of spontaneous appearance of streptomycin resistant mutants. Microscopic examination indicated that the transformants exhibited the morphological features of Anacystis species. The transformants proved stable on repeated transfer onto streptomycin supplemented media. The addition of DNAase and RNAase and both together reduced transformantion efficiency, suggesting that both DNA and RNA are necessary for transformation. Intergeneric transfer of rifampicin resistance from Rif^r A. nidulans to wild-type G. alpicola was also obtained although the frequency of transfer was relatively low: 7.5×10^{-7}. This was, however, 1.2×10^2-fold higher than the spontaneous mutation rate of rifampicin resistance in Gloeocapsa. This work represented the first demonstration of intergeneric transformation in blue-green algae and it has considerable interest, for example, markers for nitrogen fixing ability may be transferable between blue-green algae species or genera. Work is now continuing and the effects of radiation on the efficiency of transformation are being studied in detail.

In order to study the effects of radiation on genetic transduction in blue-green algae it was first necessary to demonstrate the existence of this method of genetic exchange using G. alpicola and A. nidulans. It is believed that transduction has tentatively been demonstrated using A. nidulans and cyanophage AS-1. Further work is continuing on the effects of radiation on the efficiency of this transduction process.

Buckley, C.E. and Houghton, J.A. (1975). The repair of UV induced damage in Gloeocapsa alpicola. Proc. Soc. Gen. Microbiol., 2, 83.

Buckley, C.E. and Houghton, J.A. (1976). A study of the effects of near UV radiation on the pigmentation of the blue-green alga Gloeocapsa alpicola. Archives fur Mikrobiologie, (In press).

Contractors:

1. M.R.C. Cell Mutation Unit, University of Sussex, United Kingdom.
2. The Medical Biological Laboratory, T.N.O., Rijswijk, The Netherlands.
3. Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands.
4. Institute of Radiation Genetics and Chemical Mutagenesis, University of Leiden, The Netherlands.

Contract No.: 123-74-1-BIOC

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.

Normal mammalian cells can repair damage induced in their DNA by either UV-light, ionizing radiation or chemicals. In bacteria, at least three dark repair systems have been characterized, excision repair, recombination repair (pre- and post-replicational) and a minor error-prone process that appears to be responsible for the generation of most induced mutants. In mammalian cells the strongest evidence for the importance of an excision repair mechanism has come from the human hereditary skin disease xeroderma pigmentosum (XP) (Cleaver, J.E. 1970, J. Invest. Der., 54, 181-195). Cells from XP patients seem to be defective in an initial step of DNA repair. By cell fusion techniques evidence was obtained suggesting different mechanisms were involved in different XP complementation groups (de Weerd-Kastelein, E.A. Keijzer, W., and Bootsma, D., 1972, Nature New. Biol. 238, 80-83) but the steps of DNA repair affected by the different XP mutations have not yet been identified.

There is also evidence that normal mammalian cells have a repair mechanism which acts during or after normal DNA replication in S-phase (Lehmann, A.R., 1972, J. Mol. Biol. 66, 319-337; Buhl, S.N., Stillman, R.M., Setlow, R.B., and Regan, J.D. 1972, Biophys. J., 12, 1182-1191). At the commencement of this project, however, no mutant cell lines defective in post-replication repair had been identified.

The advances that have been made in the study of DNA repair processes in bacterial cells are in large measure due to the facility with which repair-deficient strains can be made and selected. It is the object of this contract to collect, isolate or construct mutants of

mammalian cells with DNA repair deficiencies, particularly in excision repair and post-replication repair and in systems involved in repair of ionizing radiation damage, and to use the combined resources of the four participating laboratories to characterize these mutants as fully as possible both genetically and biochemically.

In our first annual report we described the characterization of five different types of human cell mutants deficient in excision repair and of the first human mutants deficient in post-replication repair. In all cases these deficiencies resulted in the syndrome xeroderma pigmentosum. In addition, evidence was obtained for differences in excision repair between human, chicken and Chinese hamster cells.

We have now identified and characterized a repair defect in cells from patients suffering from the congenital disease ataxia telangiectasia. These cells are highly sensitive to ionizing radiation but not UV. Some progress has been made in choracterization of the biochemical defect which appears to be in a prereplicational DNA repair pathway.
A further UV-sensitive strain of human cells has been isolated from a male child with sunlight sensitive skin. The cells appear to be normal in all repair functions so far studie which distinguishes them from any known xeroderma pigmentosum type.

Results of Project No. 1:

Heads of project and scientific staff: Dr. C.F. Arlett, Sussex
Professor D. Bootsma, Rotterdam
Dr. E.A. de Weerd-Kastelein, Rotterdam

Title of project: Isolation of radiation-sensitive mutants

We have now established a collection of human and rodent cell strains with proven or indications of deficiencies in the repair of DNA.

Chinese Hamster

A collection of 9 cell strains with differing UV sensitivities and different levels of DNA repair synthesis have been established.

Human

Representative cell strains of all complementation groups amongst excision deficient XP are available. In addition we have a collection of 5 excision proficient XP cell strains. Cell strain 11961 which also shows 254nm UV sensitivity but no defect in any repair process so far studied is available. A set of 23 cell strains from individuals presenting with a range of symptoms from psoriasis through sun sensitivity to multiple skin carcinoma and in various combinations have been collected.

Five cell strains from ataxia telangiectasia individuals have been collected together with representatives of the various progeric syndromes. (Hutchinson-Gilford, Hallermann-Streiff and Werner). We have now assembled representative cell strains (and some heterozygotes) of Fanconi's anemia, porokeratosis of Mibelli, disseminated superficial actinic porokeratosis, retinoblastoma, basal cell naevus and Mendes da Costa syndrome.

Publications:

Bootsma, D. and E.A. de Weerd-Kastelein (1975). Excision repair in human cells. Proc. XI International Cancer Congress, Florence 1974. Excerpta Medica International Congress Series no. 349, vol. 1, 164-169.

Cleaver, J.E., D. Bootsma, and E. Friedberg (1975). Human diseases with genetically altered DNA repair processes. Genetics, 79, 215-225.

Results of Project No. 2:

Heads of project and scientific staff : Dr. A.R. Lehmann, Sussex
Dr. P.H.M. Lohman, Rijswijk
Dr. G. Veldhuisen, Rijswijk
Dr. R.R. Hewitt, Rijswijk
Dr. S. Bachetti, Rijswijk
Dr. M.M. Abboud, Sussex

Title of project: Biochemical characterization of radiosensitive mutants

Excision repair of UV damage

In different human cells the amounts of repair replication and of excision of pyrimidine dimers are well correlated. In comparison no such correlation exists in hamster or chick cells. The level of repair replication, as compared to human cells, is much higher than the level of dimer excision, the latter being in general very low in rodent and chick cells. This points to the existence of an excision repair process in hamster and chick cell which acts on non-dimer photo products. This process is being studied in hamster lines of differing UV sensitivity.

Excision repair of γ-ray induced damage

Human cells can repair single strand breaks, double strand breaks and base damage induce in their DNA by ionizing radiation. Fibroblasts from patients with ataxia telangiectasia (A are sensitive to the lethal effects of γ-irradiation (see project No. 3). These cells have normal rates of rejoining of both single and double-strand breaks. The excision of an as yet uncharacterized type of base damage, produced preferentially by irradiation in anoxic conditions is defective in AT cells. The amount of γ-ray-induced repair replication is als reduced as compared with normal cells. This defect probably gives rise to the enhanced radiosensitivity of AT in cultured cells and in vivo.

Post-replication repair of UV damage

Cells from most patients with xeroderma pigmentosum are defective in excision-repair of UV damage. One class (XP variants) have normal levels of excision repair but a defect in post-replication repair. These studies have now been extended. Five XP variants show the extreme defect in post-replication repair. Cells from XP complementation groups A, B, C and D have a post-replication repair of intermediate magnitude. Cells from XP complementation group E and from patients with a variety of other disorders (sun sensitivity, multiple malignancies, hereditary disorders with a possible radiosensitive component) were all normal in post-replication repair, showing that the defect is specific to XP cells.

Publications:

Lehmann, A.R., S. Kirk-Bell, C.F. Arlett, M.C. Paterson, P.H.M. Lohman, E.A. de Weerd-Kastelein, and D. Bootsma (1975). Xeroderma pigmentosum cells with normal levels of excision repair have a defect in DNA synthesis after UV irradiation, Proc. Nat. Acad. Sci., 72, 219-223.

Cleaver, J.E. and D. Bootsma (1975). Xeroderma pigmentosum biochemical and genetic characteristics. Annual Review of Genetics 7.

Paterson, M.C., and P.H.M. Lohman (1975). Use of enzymatic assay to evaluate UV-induced DNA repair in human and embryonic chick fibroblasts and multinucleate heterokaryons derived from both, in Molecular Mechanisms for Repair of DNA part B (Eds. P.C. Hanawalt and R.B. Setlow) pp. 735-745.

Results of Project No.3:

Heads of project and scientific staff: Dr. C.F. Arlett, Sussex
Professor D. Bootsma, Rotterdam
Dr. J.W.I.M. Simons, Leiden
Dr. E.A. de Weerd-Kastelein, Rotterdam

Title of project: Genetic studies on DNA repair mutants

Cell fusion experiments

Several new xeroderma pigmentosum strains have been established from skin biopsies of patients and were characterized by complementation analysis. A strain from a patient in Iran (XP1TE) was assigned to complementation group C. From Dr. J.M. Parrington, Londc we obtained the strain XP8LO, derived from an XP patient with mild symptoms of the disease. The residual activity of UDS in these cells was 30-40% of control cells. This strain was assigned to complementation group A by cell fusion. It presents an interesting exception in the group A class of strains because of its high residual UDS activity and th absence of mental defects in the patient.

A complementation test has been devised for XP variants, defective in post-replication repair. The first results indicate that two clinically distinct variants XP30RO and XP4BE are in the same complementation group.

A detailed study of complementation between chicken cells and various types of human fibroblasts has been carried out. Excision repair has been observed in XP nuclei belonging to complementation group A, B and C when present in UV exposed heterokaryons with chick erythrocytes. Preliminary evidence is obtained that this excision repair does not res in the removal of thymine dimers, indicating that chicken cells do not possess the enzyme which is defective in these 3 XP groups.

Experiments to characterize the repair processes by means of the UV endo test and UDS i Chinese hamster-human hybrids having retained different numbers and types of human chromosomes are in progress. It is expected that these investigations will yield information on the localization of genes involved in DNA repair on human chromosomes.

Survival studies

We have now available three classes of UV sensitive human cells.
1. Excision deficient XP which are very UV sensitive. An absolute correlation between residual level of UDS and survival has not been demonstrated. 2. Excision proficient XP variants which may be only slightly sensitive but whose sensitivity can be enhanced by the addition of caffeine to the post-irradiation medium and 3. Strains 11961, where there is substantial UV sensitivity but, as yet, no defect recognized in any repair process for UV damage. All other strains have given an uniform response.

The first example of a human mutant sensitive to ionizing radiation has been shown by the response of cells from ataxia telangiectasia to Y radiation. Unlike the response to UV we have found some variation amongst wild-type cells to Y rays. Cells from the Hutchinson-Gilford progeria syndrome also show some indications of an enhanced sensitivity.
Cross sensitivity studies have shown that UV sensitive cells are not sensitive to irradiation and vice-versa. The response to other DNA damaging agents have failed, as yet, to demonstrate any sensitivity to mitomycin C, although there are strong indications that excision defective XP cells are sensitive to 310nm wave length UV light.

Mutation studies

UV induced mutant frequencies for hypoxanthine-guanine-phosphoribosyl-transferase (HGPRT)-deficiency were 2.2×10^{-6}/erg in wild type cells and 55×10^{-6} in XP TEKO (preliminary assigned to complementation group D). When the mutant frequencies are plotted against survival the difference between both cell strains disappears. A method has been developed for liquid holding of human fibroblasts. Cells are kept in a stationary phase by contact inhibition for about a week. Only 4-10 percent of the cell population undergoes cell division during this period. It was shown that during liquid holding UV- induced damage in both wild type cells and XP cells is largely repaired as measured by cell survival. After liquid holding the mutant frequency in UV- irradiated XP cells is not any more enhanced indicating that this repair is error-free.

Ouabain resistance has been shown to be a non-mutable locus with Y irradiation using Chinese hamster and mouse lymphoma (L5178Y) cells. As a consequence of our interest in the sensitive ataxia cells we have shelved our plans for developing ouabain resistance as an alternative selective system with human cells and have returned to the use of the selective system for azaguanine-resistant (HGPRT, negative) cells. In an attempt to avoid frequent and costly medium changes the use of bulk culture vessels for mutation assay is under assessment.

Publications:

Arlett, C.F., S.A. Harcourt and B.C. Broughton (1976). The influence of caffeine on cell survival in excision-proficient and excision-deficient xeroderma pigmentosum and normal human cell strains following ultraviolet light irradiation, Mutation Res. 33, 341-346.

Arlett, C.F., D. Turnbull, S.A. Harcourt, A.R. Lehmann and C.M. Colella (1975). A comparison of the 8-azaguanine and ouabain resistance systems for the selection of induced mutant Chinese hamster cells, Mutation Res., 33, 261-278.

Taylor, A.M.R., D.G. Harnden, C.F. Arlett, S.A. Harcourt, A.R. Lehmann, S. Steve and B.A. Bridges (1975). Ataxia telangiectasia: a human mutation with abnormal radiatio sensitivity, Nature, 258, 427-429.

Van Zeeland, A.A. and J.W.I.M. Simons (1975). The effect of calf serum on the toxic of 8-azaguanine. Mutation Res., 27, 135-138.

Van Zeeland, A.A. and J.W.I.M. Simons (1975). Ploidy level and mutation to hypoxan guanine phosphoribosyl transferase (HGPRT) deficiency in Chinese hamster cells. Mutation Res., 28, 239-250.

Van Zeeland, A.A. and J.W.I.M. Simons (1976). The use of correction factors in the determination of mutant frequencies in populations of human diploid skin fibroblasts. Mutation Res., in the press.

Knaap, A.G.A.C. and J.W.I.M. Simons (1975). Mutational assay system for L5178Y mouse lymphoma cells, using hypoxanthine guanine phosphoribosyl transferase (HGPRT)-deficiency as marker. The occurrence of a long expression time for mutations induced by X-rays and EMS. Mutation Res. 30, 79-110.

Van Zeeland, A.A. (1975). Resistance to purine analogues. Studies of its suitability in mutation research with mammalian cells in vitro. Thesis, State University Leiden.

Bootsma, D., E.A. de Weerd-Kastelein, W.J. Kleijer and W. Keijzer (1975). Genetic complementation analysis of xeroderma pigmentosum, in Molecular Mechanisms for Repair of DNA, Part B (Eds. P.C. Hanawalt and R.B. Setlow) pp. 725-728, Plenum Press: New York.

Bootsma, D. (1976). Genetic aspects of DNA repair mechanisms in mammalian cells. Proc. of the Princess Takamatsu Cancer Research Fund, University of Tokyo Press, in the press.

Kraemer, K.H., E.A. de Weerd-Kastelein, J.H. Robbins, W. Keijzer, S.F. Barrett, R.A. Petinga and D. Bootsma (1975). Five complementation groups in xeroderma pigmentosum. Mutation Res. 33, 327-340.

Contractor: The University College of Swansea.
Contract No: 119-72-1 BIO U.K.
Dr. James M. Parry

Studies of the genetic, molecular and adaptive properties of RAD loci in yeast.

Bacterial and viral systems have provided much valuable information in the development of our understanding of the hereditary effects of radiations. However, a similar understanding of such processes in mammalian cells is fraught by a great many difficulties not the least of which is the financial one stemming from the cost of much of the research involving tissue culture techniques. There is thus an obvious need to utilize organisms of intermediate complexity if we are to understand the processes of DNA repair and genetic change in chromosomal organisms.

The simple eucaryote, Saccharomyces cerevisiae provides an ideal material for the study of DNA repair and the processes of genetic change which occur after radiation and chemical mutagen treatments. Our research programme in 1975 involved a number of projects which were intended to provide us with basic information on the effects of radiation and chemical mutagen damage in yeast.

During the year we have been studying the influences of cell division and inhibitors of repair upon the fundamental processes of genetic change. These studies have resulted in the development of a model of induced recombination and some interesting concepts of the nature of mitotic non-disjunction in yeast. Considerable advances have been made in the development of biochemical techniques for the study of DNA repair in yeast and our efforts will be increased in this direction in the future.

Results of Project No.1.

Head of Project and Scientific Staff. Dr. James M. Parry.

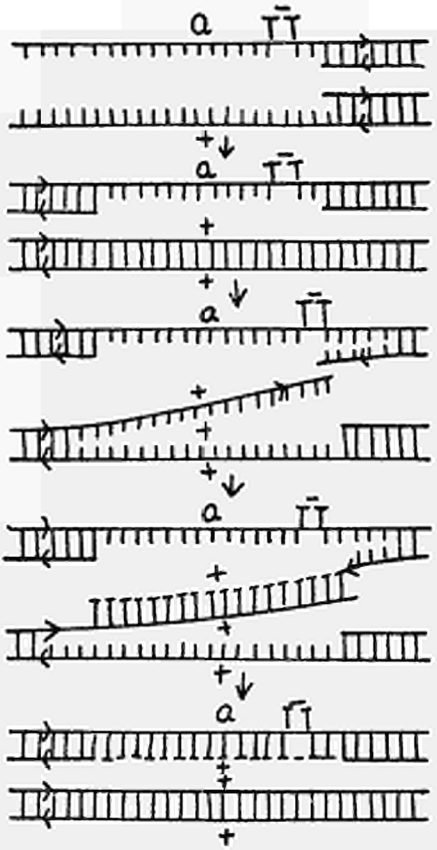
Title of Project: The development of a model for the mechanism of mutagen induced mitotic gene conversion in yeast.

The exposure of diploid cultures of the yeast Saccharomyces cerevisiae to physical and chemical mutagens may result in the induction of mitotic recombination both within (intra) and between (inter) genes. The intragenic event generally occurs by gene conversion, a process characterised by its failure to yield reciprocal products during recombination and may be scored by the formation of prototrophic convertants in auxotrophic heteroallelic yeast cultures. Intergenic recombination or crossing-over yields reciprocal products of genes and may be detected by the formation of recessive homozygous colonies in a heterozygous culture.

The published evidence indicates that induced mitotic gene conversion is intimately associated with DNA repair mechanisms. Several models have been proposed to account for meiotic gene conversion but such models will not account for the mitotic process. Unlike meiotic conversion the mitotic event is not polarised and is not associated with crossing-over of outside markers.

We have developed a model of induced gene conversion which is illustrated below. The model attempts to describe UV induced gene conversion produced by de novo DNA synthesis during post-replication repair and occurring without crossing-over of outside markers, only two of the four DNA strands involved are shown in the figure.

The model assumes that a UV-induced pyrimidine dimer lies within a region of DNA differing in the bases at one site. DNA replication within a replicon results in a daughter-strand gap opposite a pyrimidine dimer. Daughter strands of opposite polarity derived from the 2 DNA duplexes associate to form a temporary pairing structure with de novo DNA synthesis on the intact strand. Separation of the temporary pairing configuration is followed by a reassociation of parental and daughter strands. DNA ligase action results in the attachment of newly synthesised DNA to the strand derived from the incomplete helix. The single-stranded region has now been eliminated from the DNA duplex containing the pyrimidine dimer with the formation of a region of mismatched bases.



Excision-repair of the pyrimidine dimers may now lead to the correction of the mismatch and thus produce an observable conversion event. In a mitotic cell, conversion may also result from segregation of the wild type DNA strand without the correction of the mismatch in the heteroduplex. We presume that the mitotic segregation of strands accounts for the increase in gene conversion observed in excision-deficient strains of yeast.

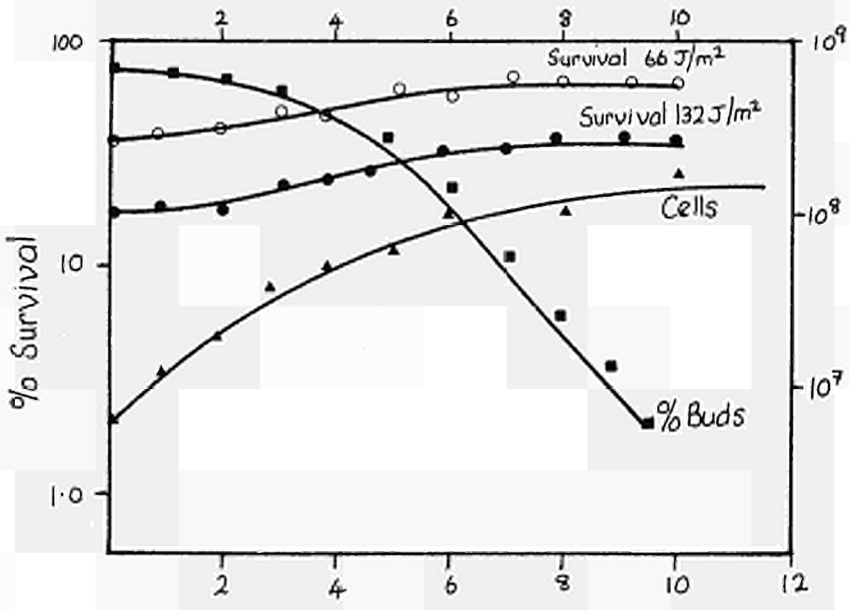
The model may also account for gene conversion induced by other inactivating agents such as ionising radiation and chemical mutagens. All these agents produce DNA base damage and it is probable that similar post-replication gaps are formed opposite such damaged bases.

Results of Project No.2.

Head of Project and Scientific Staff. Dr. James M. Parry,
Dr. Elizabeth M. Parry &
Dr. P. J. Davies.

Title of Project: The variation in UV sensitivity of yeast cultures during cell growth.

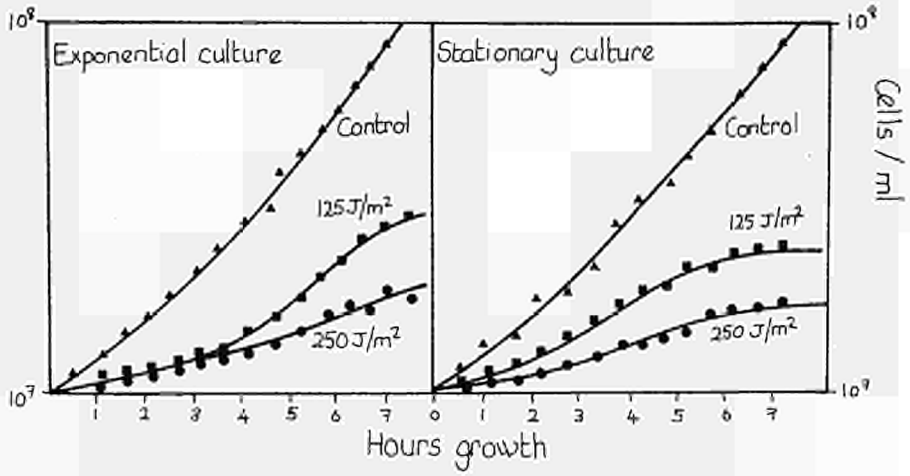
Wild type and excision-defective strains of yeast show characteristic responses to UV damage during the various stages of cell growth. During the exponential phase of growth the cells show maximum cell resistance to UV light treatment and irradiated cultures innoculated into fresh medium are characterised by a rapid entry into cell multiplication. In contrast, cells which have entered the stationary phase show increased sensitivity to UV damage and irradiated cultures innoculated into fresh medium show a growth delay of $1\frac{1}{2}$ to 2 hours before cell multiplication takes place. In a previous report we described a detailed study of the changes in sensitivity in cell sensitivity and macromolecular syntheses during the transition period from exponential to the stationary phase of growth.



We have examined the growth response to UV light of a wide range of yeast mutants. The diploid X-ray sensitive mutant $\frac{rad_{50}}{rad_{50}}$ has been studied during the transition period between the exponential and the stationary phase of cell growth. The sensitivities of this culture after UV exposures of $66J/M^2$ and $132J/M^2$ are shown in Figure I.

The results presented demonstrate that in the $\underline{rad_{50}}/\underline{rad_{50}}$ culture no increases in UV sensitivity were detectable as the culture progresses from the exponential to the stationary phase. In fact at the two UV doses utilized small increases in cell resistance were detectable over a period of 4 to 9 hours growth.

Exponential and stationary phase cultures of $\underline{rad_{50}}/\underline{rad_{50}}$ were exposed to 125 and 250 J/M^2 of UV light and re-innoculated into fresh medium. The effects of UV light upon cell division in both exponential and stationary phase cultures are shown in Figure 2.



The figure demonstrates that after UV irradiation of $\underline{rad_{50}}/\underline{rad_{50}}$ both exponential and stationary phase cells showed a UV induced growth delay of $1\frac{1}{2}$ to 2 hours before significant increases could be detected in cell numbers.

The results of the experiments with diploid cultures of $\underline{rad_{50}}/\underline{rad_{50}}$ implicate a product of the $\underline{RAD_{50}}$ gene in the UV resistance of exponential phase cultures and in the abolition of the growth delay found after UV irradiation of exponential phase cells of wild type and excision deficient mutants of yeast.

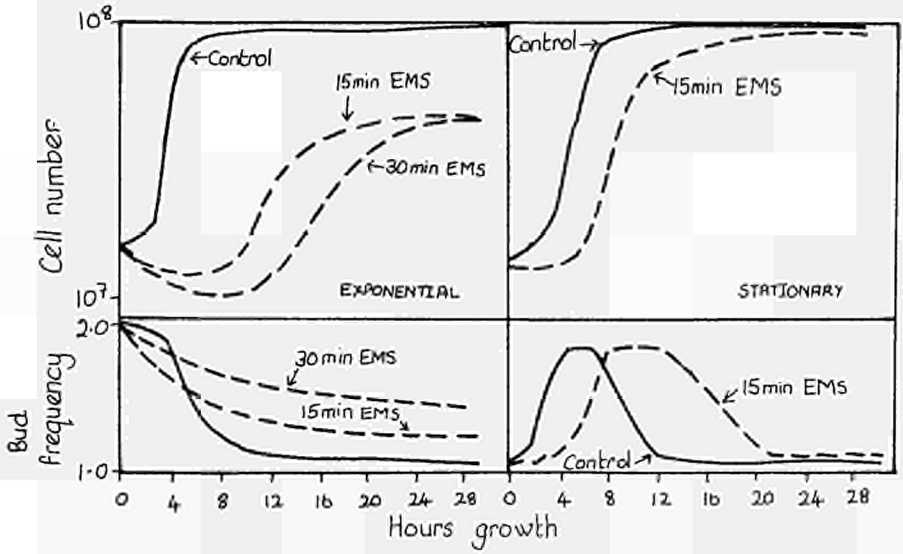
Results of Project No.3.

Head of Project and Scientific Staff. Dr. James M. Parry and
Dr. Elizabeth M. Parry.

Title of Project: The sensitivity of yeast cultures to chemical mutagens during cell division.

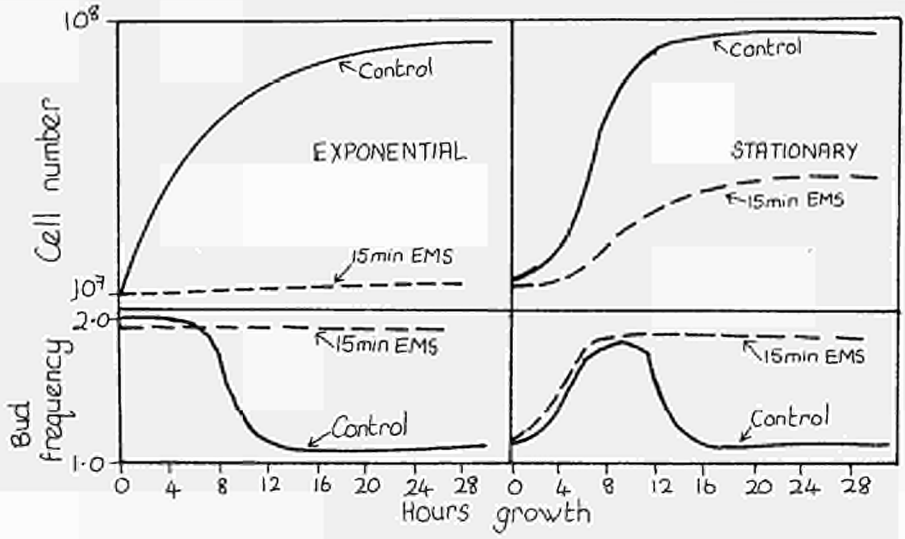
Unlike their response to UV light and ionising radiation, yeast cultures show maximum cell sensitivity to chemical mutagens during the exponential phase growth with a significant increase in resistance as the cultures enter stationary phase. We have investigated these important differences in sensitivity further by studying the effects of chemical mutagens upon macromolecular synthesis in cultures of different cell age derived from yeast strains carrying wild type and radiation sensitive alleles.

The effects of 2% ethyl methane sulphonate upon cell division and the frequency of budding cells in exponential and the stationary phase cultures of wild type yeast reinnoculated in fresh medium are shown in Figure I.



Control cultures of both exponential and stationary phase cells rapidly enter cell division and complete their phase of growth between 7 to 10 hours after reinnoculation. After 4 hours both cultures complete their budding cycle and contain mainly single cells after 10 hours. As can be seen in Figure 1 after EMS treatment the yield of cells is considerably reduced in exponential compared to stationary cultures. In the sensitive exponential cells the frequency of budding cells is reduced during the period of cell division but does not reach unity in either of the treated cultures. In the more resistant stationary phase cells the initiation of cell budding is delayed by approximately three hours but although delayed the frequency of cells reaches unity after approximately 20 hours.

In view of the culture age response of the $\underline{RAD_{50}}$ gene to UV light described in the previous report we have investigated the effects of chemical mutagens upon $\underline{rad_{50}}/\underline{rad_{50}}$ cultures. The response of exponential and stationary phase cells of $\underline{rad_{50}}/\underline{rad_{50}}$ to 2% EMS are shown in Figure 2.



Control cultures of $\underline{rad_{50}}/\underline{rad_{50}}$ behave in an essentially similar manner to that as wild type cells, but there are a number of significant differences in the response of the cells after treatment with ethyl methane sulphonate. At identical EMS concentrations no cell growth occurs in treated exponential plane cells and there were no detectable changes in the frequencies of budding cells. Mutagen treated stationary phase cells enter cell multiplication after approximately 3 hours with an approximately 3 fold increase in cell numbers. During this period of cell multiplication cell budding increases until after 4 hours each cell has a mean of one bud. This frequency remains constant for the remainder of the treatment period. The observations indicate that irrespective of the culture stage the cells of $\underline{rad_{50}}/\underline{rad_{50}}$ are unable to proceed beyond the DNA synthetic period.

These studies are now being extended to include investigations of macromolecular syntheses after ionising radiation treatment and combined exposure to radiations and chemical mutagens.

Results of Project No.4

Head of Project and Scientific Staff: Dr. James M. Parry
Mrs. Margaret Clatworthy

Title of Project: The induction of mitotic chromosome non-disjunction in yeast.

The diploid yeast strain D_6, produces red colonies and is sensitive to cycloheximide at a concentration of 2 ppm. The loss of one copy of chromosome VII by mitotic chromosome non-disjunction results in a monosomic cell (2n-1), which produce colonies that are capable of growth on complete medium containing cycloheximide. We have previously reported the effects of gamma irradiation and heat shock at 52° upon the induction of monosomic colonies in yeast. These studies have been extended to include an analysis of the response of D_6 to UV light treatment.

Stationary phase cultures of D_6, irradiated with UV light and plated immediately upon medium containing cycloheximide show little or no increase in the frequency of white cycloheximide resistant colonies. Thus, as with both gamma irradiation and heat shock the detection of monosomic colonies requires an experimental design which allows for the "expression" of the induced damage before the application of the selective agent.

UV light exposed cultures of D_6 were therefore innoculated into non-selective medium for periods of 48 hours in the dark, before plating upon complete medium containing 2 ppm cycloheximide. The frequencies of white cycloheximide resistant colonies produced after 0 to 650 Joules/M^2 of UV light are shown in Table 1.

Table 1.

Treatment (UV)	Final yield of viable cells/ml x10^8	Frequency of white cyc^r colonies/10^5 cells	Treatment (UV)	Final yield of viable cells/ml	Frequency of white cyc^r colonies/10^5 cells
Control	2.4	5.8 ± 1.1			
	1.8	4.0 ± 0.9			
	2.8	5.6 ± 1.1			
132 Joules UV	1.9	29.2 ± 2.4	132 Joules UV + Pr light	2.6	22.4 ± 2.1
	2.0	27.0 ± 2.3		2.9	15.8 ± 1.8
	2.1	28.4 ± 2.4		2.1	17.3 ± 1.9
264 Joules	1.9	64.8 ± 3.6	264 Joules + Pr light	1.9	46.0 ± 3.0
	1.8	65.8 ± 3.6		2.0	35.2 ± 2.7
	2.0	58.4 ± 3.4		1.9	37.3 ± 2.7
400 Joules	1.9	21.5 ± 3.2	400 Joules + Pr light	2.2	29.7 ± 2.4
	1.9	48.7 ± 3.1		2.3	39.2 ± 2.8
	1.6	63.3 ± 3.5		2.1	36.4 ± 2.7
530 Joules	2.3	56.3 ± 3.4	530 Joules + Pr light	1.9	27.3 ± 2.3
	1.9	57.9 ± 3.4		1.7	26.4 ± 2.3
	2.0	59.6 ± 3.5		1.9	28.4 ± 2.4
650 Joules	1.0	56.4 ± 3.4	650 Joules + Pr light	1.5	26.2 ± 2.3
	1.3	50.9 ± 3.2		1.4	28.5 ± 2.4
	1.1	68.7 ± 3.7		1.7	32.3 ± 2.5

The results in Table 1. demonstrate that UV light treatments above 132 J/M^2 produce significant increases in the frequency of presumptive monosomic colonies in all the 3 replicate experiments performed. Also of interest in Table 1. are the effects of exposure to visible light treatment after UV exposure and before growth in non-selective medium. The results demonstrate that the photoreactivating light treatment produced a reduction in the frequency of monosomic colonies at all the UV light exposures utilized. For example after 264 J/M^2 of UV light, photoreactivating light treatment produced a reduction of monosomic colonies from 63.0 to $39.5/10^5$ viable cells. Exposure to visible light treatment before UV exposure had no effect upon the frequency of monosomic colonies. We are thus led to the conclusion that photoreactivating light sensitive lesions i.e. pyrimidine dimers are implicated in the induction of monosomic colonies by mitotic non-disjunction.

In view of the involvement of purimidine dimers in the induction of monosomy in yeast we are now actively investigating the effects of the well known repair deficiency loci (rad) upon mitotic chromosome non-disjunction.

Results of Project No. 5.

Head of Project and Scientific Staff: Dr. James M. Parry and
Mrs. Margaret Clatworthy.

Title of Project: Variation in DNA content of spontaneous and UV light induced monosomic colonies in yeast.

As described in the previous report, the yeast strain D_6 has been used to detect the production of monosomic colonies produced by chromosome non-disjunction. We have performed a comparative study of the DNA content of individual cells of the parental red, cycloheximide sensitive colonies and the white cycloheximide resistant monosomic colonies isolated from both control and UV treated cultures of D_6. The DNA content, expressed in mgms/10^8 cells estimated by the diphenylamine reaction are shown in Table 1.

The results obtained, demonstrate that the DNA contents of the 10 red cycloheximide sensitive colonies derived from control plates vary from 4.0 to 4.6 mgms/10^8 cells with a mean of 4.3 mgms/10^8 cells. The 10 red cycloheximide sensitive colonies isolated from cycloheximide containing media after UV treatment show closely similar DNA content, varying from 3.9 to 4.6 mgms/10^8 cells with an identical mean of 4.3 mgm/10^8 cells. The variations of the individual colonies of both groups of cells compare closely with that of the standard diploid strains utilized in our laboratory.

In contrast, the white cycloheximide resistant colonies derived from both untreated and UV light exposed cultures show a significant reduction in DNA content per cell. The 20 individual white cycloheximide resistant colonies produced spontaneously vary in DNA content from 3.1 to 4.2 mgm/10^8 cells. The mean DNA content per cell was 3.7 mgms/10^8 cells, which represents a 13.3% reduction in DNA content compared to the parental D_6 culture. Cultures 4, 5, 6, 10, 15 and 16 which show DNA contents close to that of the parental strain are capable of sporulation on acetate medium. None of the remaining cultures undergo sporulation.

The 10 white cycloheximide resistant cultures isolated after UV treatment show increased variation in DNA content from 2.4 to 4.0 mgms/10^8 cells with a mean DNA content of 3.23 mgms/10^8 cells.

This value represents a mean reduction in DNA content of 24.5%. Only one of the cultures i.e. No.3 had a DNA content close to that of the parental D_6 culture and this was the only culture capable of undergoing sporulation on acetate medium. In spite of the fact that a number of the cultures show DNA contents close to that of stock haploid cultures e.g. No.1, none of the cultures undergo mating with either a or α haploid strains.

The reductions in DNA content found amongst the presumptive monosomic colonies are much greater than would be expected from the loss of a single copy of chromosome VII. The results suggest that in this strain of yeast the loss of chromosome VII was accompanied by the loss of material from other chromosomes during mitotic chromosome non-disjunction and that this chromosome loss was quantitatively greater after UV exposure.

TABLE 1.

				UV treated 500J/M^2				Spontaneous			
Strain	DNA mgms/10^8 cells	D_6 red cyc^s	DNA mgms/10^8 cells	D_6 white cyc^r leu^-trp^- monosomic	DNA mgms/10^8 cells	D_6 red cyc^r	DNA mgms/10^8 cells	D_6 white cyc^r leu^-trp^- monosomic	DNA mgms/10^8 cells	D_6 white cyc^r leu^-trp^- monoso	DNA mgms/10^8 cells
		1.	4.2	1.	2.8		4.3	1.	3.6	11.	3.8
n	2.4	2.	4.4	2.	2.4		4.4	2.	3.3	12.	3.7
2nJ	4.6	3.	4.6	3.	4.0		4.0	3.	3.9	13.	3.8
JD1	4.4	4.	4.2	4.	3.8		4.6	4.	4.2	14.	3.3
		5.	4.0	5.	2.9		4.5	5.	4.0	15.	4.0
3n	6.7	6.	4.1	6.	3.1		4.6	6.	4.1	16.	3.9
4n	8.5	7.	4.3	7.	3.2		3.9	7.	3.7	17.	3.7
		8.	4.1	8.	3.5		4.2	8.	3.6	18.	3.2
2nL	3.2	9.	4.6	9.	2.9		4.3	9.	3.5	19.	3.6
		10.	4.3	10.	3.7		4.1	10.	4.1	20.	3.1

Results of Project No. 6.

Head of Project and Scientific Staff: Dr. James M. Parry and Dr. W. E. Evan

Title of Project: The cross-sensitivity of heat sensitive mutants to radiations and chemical mutagens.

The mutants of yeast XS1, XS2, XS2-1, XS2-2 and XS-3 were isolated by a number of workers on the basis of their sensitivity to ionising radiations. All five of these mutants were cross-sensitive to UV light, nitrous acid, ethyl methane sulphonate and heat treatment at 52°C. XS2-1 and XS2-2 also show increased cell death when stored in non-nutrient solution after UV light and heat treatment (negative liquid holding).

Seventeen yeast mutants HS1 to HS-17 were isolated on the basis of their sensitivity to heat shock at 52°. Of the 17 mutants, 15 were dominant or partially dominant in heterozygous diploids, the other two being recessive. The mutants are thus unlike the classical rad loci of yeast which are predominantly recessive when combined in heterozygous diploids with the wild type RAD allele.

Because of their unusual characteristics we have investigated the cross-sensitivity of the HS mutants to ionising radiation and ethyl methane sulphonate. A generalised outline of the pattern of sensitivity obtained is shown in Table 1.

Table 1.

Inactivating agent	Mutant Type: Mutant isolated on the basis of sensitivity to ionising radiation e.g. XS1.	Mutant isolated on the basis of sensitivity to heat e.g. HS2.	Mutant isolated on the basis of sensitivity to UV light e.g. rad_3.
Ionising Radiation	Sensitive	Sensitive only in exponential part of survival curve.	Resistant
UV light	Sensitive	Small increase in sensitivity	Very sensitive
Alkylating agents	Sensitive	Sensitive	Resistant
Heat shock at 52°	Sensitive	Sensitive	Resistant

It can be seen from the table that the HS strains represent a group of predominantly dominant mutations which confer cross-sensitivity to both ionising radiation and alkylating agents.

Wild type RAD cultures of yeast show increased sensitivity to heat shock and EMS in the exponential phase of growth compared and the stationary phase. A similar pattern of sensitivity to heat shock and EMS was shown by the HS mutants, indicating that differences in the physiological condition of the mutants does not account for the sensitivity of the HS cells.

Results of Project No.7.

Head of Project and Scientific Staff: Dr. W. E. Evans and Dr. James M. Pai

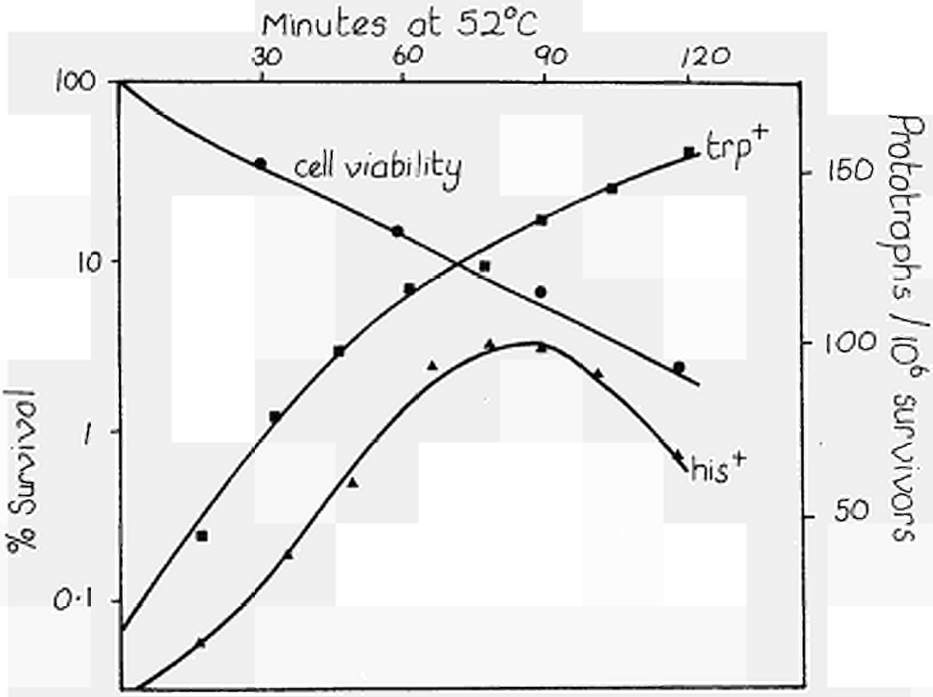
Title of Project: The induction of genetic change in yeast by elevated temperatures.

Mutants of yeast, isolated on the basis of their sensitivity to elevated temperature are all sensitive to ionising radiations and alkylating agents (see report No.6). In view of the correlation between the repair of heat and ionising radiation damage we have investigated in some detail the types and range of genetic change which may be induced by heat treatment at 52^{o} and growth at supra optimal temperatures up to 42^{o}. A range of yeast cultures were utilized which are capable of detecting mutation to drug resistance, prototrophs produced by mitotic gene conversion, recessive homozygosis produced by mitotic crossing-over and monosomic colonies produced by mitotic chromosome non-disjunction.

Mutation from cycloheximide sensitivity to resistance after heat shock at 52^{o} and storage in saline at 37^{o} was measured in haploid yeast cultures. Both treatments result in increases in mutant frequency, reaching maxima after 120 min. and 43 days at 52^{o} and 37^{o} respectively.

Mitotic crossing-over between the ade_2 gene and the centromere of chromosome XV was increased in diploid yeast cultures by heat shock at 52^{o} to reach a maximum of approximately 3% reciprocal homozygosis after heat treatment for 4 hours. Increases in homozygosis produced by mitotic crossing-over was also produced by incubation in saline at 37^{o} and by growth at temperatures from 37^{o} to 39^{o}.

Mitotic gene conversion was detected by the measurement of prototrophic colonies produced at both the tryptophan-5 and histidine-4 loci. The responses of both these loci and cell viability after heat shock at 52^{o} for up to 120 min. are shown in Figure 1.



The results in Figure 1 demonstrate that heat treatment produces significant increases in the production of prototrophic convertants at both the histidine-4 and tryptophan-5 loci, although the extent of conversion induction was greater at the tryptophan-5 locus. Similar increases in mitotic gene conversion were also detectable after incubation in saline at 37° and growth at temperatures from 37° to 39°.

Mitotic chromosome non-disjunction of chromosome VII was measured by the detection of monosomic colonies (2_{n-1}) resistant to cyclaheximide and white in colour from a red diploid culture sensitive to cycloheximide. Increases in the frequency of monosomic colonies were detectable after heat shock at 52° and after growth in nutrient medium at temperatures between 35° to 42°.

The results demonstrate that heat treatment under a range of conditions is an effective inducer of a number of genetic events in yeast and that in many respects heat treatment has similarities to the genetic changes produced by ionising radiations.

Results of Project No.8.

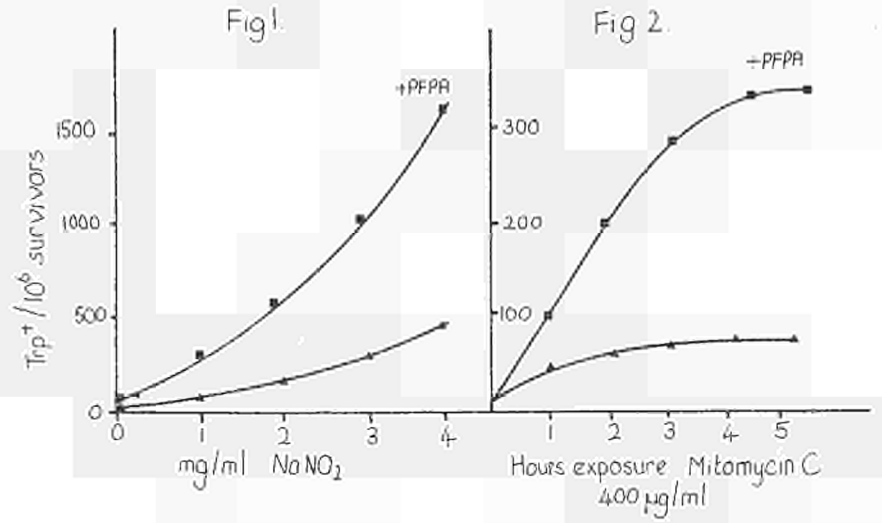
Head of Project and Scientific Staff: Dr. P. J. Davies and Dr. James M. Pa

Title of Project: The effects of p-flurophenyl alanine upon cell sensitivity of mitotic recombination in yeast.

Microbial cultures grown in the presence of the amino acid analogue p-flurophenyl alanine (PFPA) shown an enhanced mutagenic response to UV irradiation (Talmud & Lewis 1974, Johnson 1975). These results have been interpreted on the basis of PFPA incorporation into the enzymes responsible for the repair of UV damage.

Analogue sensitive diploid yeast cells, grown in the presence of 0.3 mgm/ml and 0.5 mgm/ml PFPA show reduced cell growth of 25% and 65% inhibition respectively together with significant increases in the frequency of prototrophs produced by mitotic gene conversion at both the histidine -4 and tryptophan - 5 loci.

We have investigated the additive effects of prior growth in the presence of PFPA upon cell viability and the induction of mitotic gene conversion after mutagen treatment with UV light, ethyl methane sulphonate, nitrous acid and mitomycin C. All 4 mutagens are potentiated in their effects upon mitotic gene conversion. The response of mitotic gene conversion at the tryptophan - 5 locus in yeast cells grown in the presence and absence of 0.3 mgms/ml PFPA are shown in Figures 1 and 2 for nitrous acid and mitomycin C treatment respectively.



Both figures demonstrate the increased induction of gene conversion/unit of mutagen and/surviving cell in cultures exposed to PFPA compared to the untreated control cells.

Yeast cells exposed to PFPA before mutagen treatment showed increased sensitivity to cell killing by UV and EMS treatment had little effect upon cell killing by nitrous acid and mitomycin C treatment. The potentiation of cell killing after UV treatment has been investigated further in haploid and diploid excision-defective mutants of yeast. The results of these experiments demonstrate that in excision-deficient strains there is a further potentiation of UV killing produced by PFPA treatment compared to wild type cultures.

The results indirectly demonstrate that repair enzymes other than those of excision-repair are affected by the incorporation of PFPA.

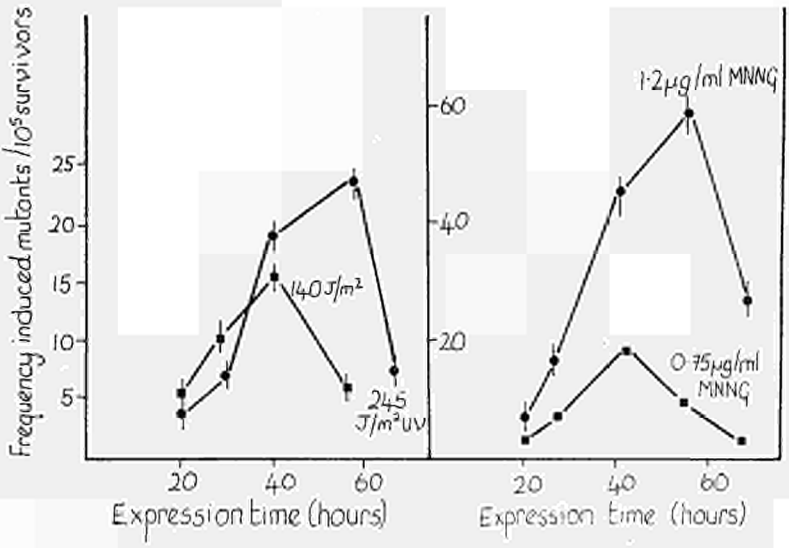
Results of Project No.9.

Head of Project and Scientific Staff: Dr. P. J. Davies and Dr. James M.

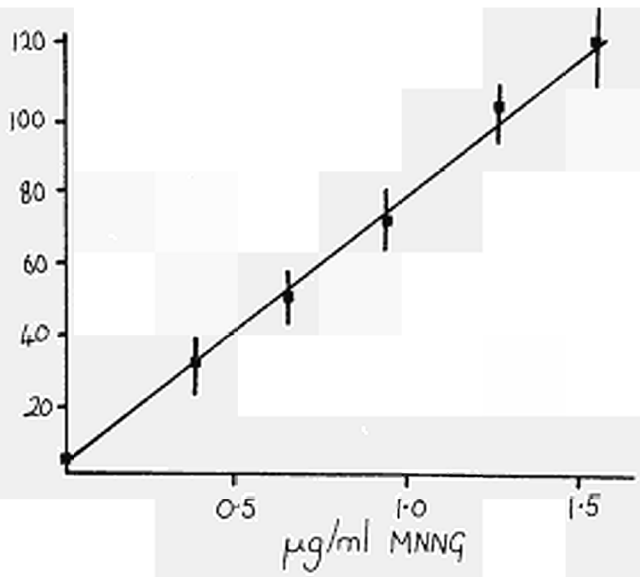
Title of Project: The induction of Ouabain resistant mutants in Chinese hamster V 79 cells.

The use of the steroid ouabain, which causes specific inhibition of the plasma membrane Na^{+} - K^{+} - Mg^{++} activated A T Pase has been described by Baker et al (1974) as a selective system for the measurement of mutation induction by physical and chemical mutagens in mammalian tissue culture cells. They demonstrated that ouabain resistant clones could be increased 40 fold by ethyl methane sulphonate but only 2 fold by N-methyl-N'-nitrosoguanidine (MNNG). In view of the high activity of MNNG in microbiol cultures we have performed a comparative study of the effects of both UV irradiation and MNNG upon the induction of ouabain resistant clones in Chinese hamster V79 cells.

The results obtained indicate that after both UV treatment and MNNG exposure the frequency of ouabain resistant clones was dependent upon the expression time between the mutagen treatment and the addition of the selective agent. The results of a typical series of experiments are shown in Figures 1 and 2 for UV light and MNNG exposure respectively.



The effects of 0–1.5 ngms/ml of MNNG at an expression time of 40 hrs upon the frequency of ouabain resistant mutants are shown in Figure 3 and are expressed in the basis of the mutant frequency per surviving cell.



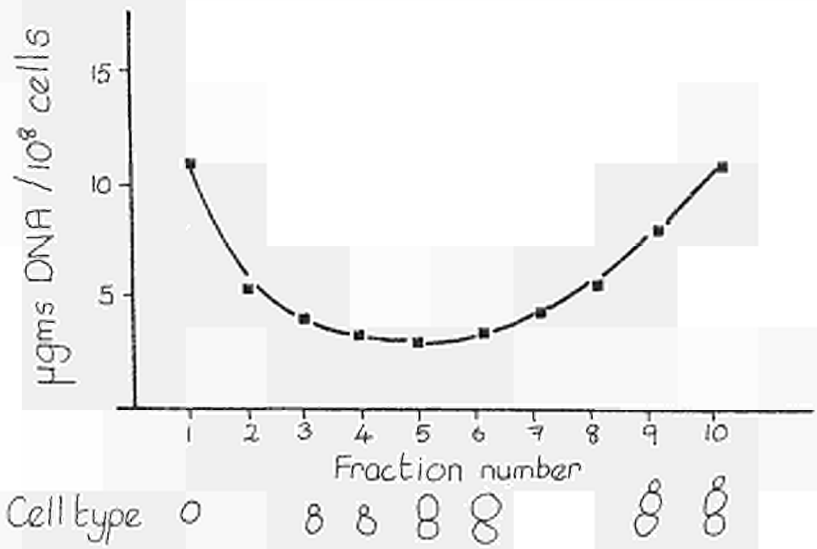
The results clearly demonstrate that under the appropriate experimental conditions MNNG was an effective mutagen in 79 cells, producing a 600 fold increase in mutation frequency at 10% survival.

Results of Project No. 10.

Head of Project and Scientific Staff: Dr. James M. Parry, Dr. P. J. Davies and Mr. R. S. Tippins.

Title of Project: Variation in mutagen induced cell lethality and mitotic recombination during the cell cycle of yeast.

Exponentially growing cultures of yeast have been separated into discrete stages of the cell cycle by the use of sucrose gradient centrifugation in a slow speed zonal rotor. A typical separation pattern is shown in Figure 1. Fraction 1 is made up mainly of small single cells in the process of DNA replication, fraction 3 and 4 single cells with buds, fractions 5 and 6 double cells and fractions 9 and 10 double cells with buds, in the process of DNA replication.



Samples of cells derived from each of the cell stages represented by the fractions from the zonal rotor were exposed to a range of treatments with UV light and nitrous acid. After mutagen treatment comparative studies were made of cell lethality, mitotic gene conversion and mitotic crossing-over.

The main points to emerge from the experiments were as follows:

1. There were two discrete periods when yeast cells were sensitive to inactivation by a number of UV doses and these stages correspond to the periods of DNA synthesis. Cell survival was increased by post UV treatment of irradiated cultures by photoreactivating light or liquid holding treatment prior to plating. The maximum response of irradiated cells to the post-treatments were observed at the most UV sensitive cell stages.

2. The response of UV induced mitotic crossing-over paralled that of cell lethality, with the maximum induction of homozygosity correlating with the periods of maximum sensitivity to inactivation.

3. The frequency of UV induced mitotic gene conversion was increased throughout the cell cycle although the maximum yield of gene conversion was observed at those stages when the cells were undergoing DNA synthesis.

4. In contrast to the response of yeast cells to UV light, the inactivation of cells by the chemical mutagen nitrous acid showed a single discrete period of sensitivity which occurred at a cell stage when DNA replication was completed and showed a correlation with the maximum frequency of budding cells.

5. Both mitotic gene conversion and mitotic crossing-over were induced by nitrous acid throughout the cell cycle with a peak of induction of both events produced at the time of maximum cell lethality.

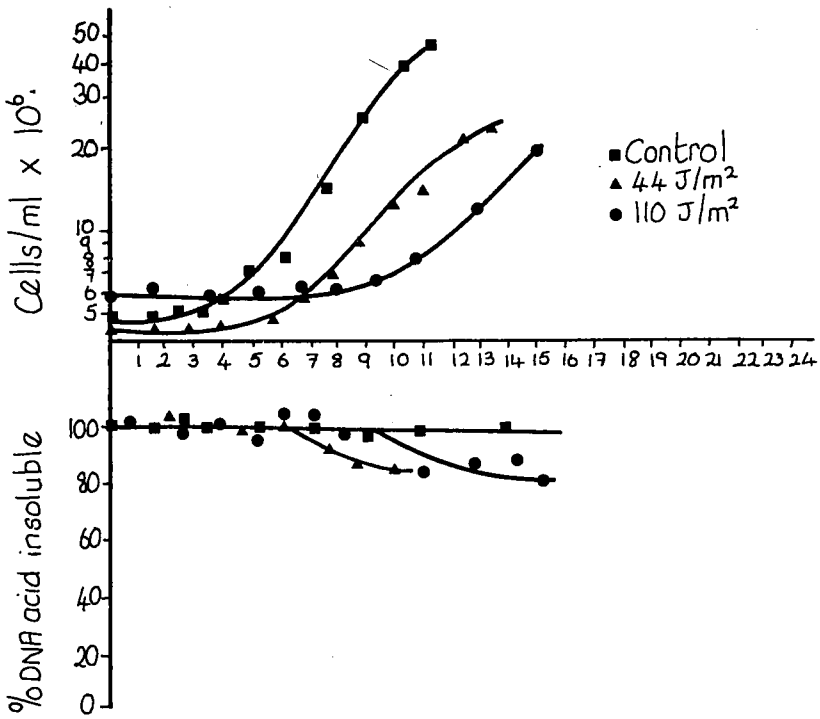
The preliminary results obtained in these experiments clearly indicate differences in the time of cell sensitivity to chemical and physical mutagens during the cell cycle, together with variations in repair activity and the induction of genetic change.

Results of Project No. 11.

Head of Project and Scientific Staff: Dr. P. Wilmore and Dr. James M. Parry

Title of Project: DNA degradation after mutagen treatment in yeast.

The yeast strain tmpl-1 supplied by Dr. M. Brendel incorporates low levels of ^{3}H-thymidine monophosphate. Cultures labelled with this precursor were exposed to the inactivating agents, UV, nitrous acid and EMS. Treated cells were assayed for survival in each case and in the case of UV and EMS, were incubated in growth medium for periods up to 20 hrs. During this period the % of radioactivity remaining in the acid insoluble DNA and the rates of cell division were determined. The results of some typical experiments are shown in Figure 1.



The results demonstrate that the mutagens produce significant delays in the initiation of growth and the rate of cell division is reduced. In the control culture, cell division commences approximately 3 hours after incubation. Treatment with 44 J/M^2 UV results in a division delay of about 5 hours.

Figure 1 also demonstrates that mutagen treatments also result in the loss of radioactive material from the acid insoluble DNA of treated cells. This degradation occurs only in dividing cells after a period of mutagen induced growth delay. Due to the considerable delay in initiation of cell division very high doses have not been used. The period of DNA degradation after mutagen treatment in yeast correlates closely with the time at which the molecular events leading to mitotic recombination take place.

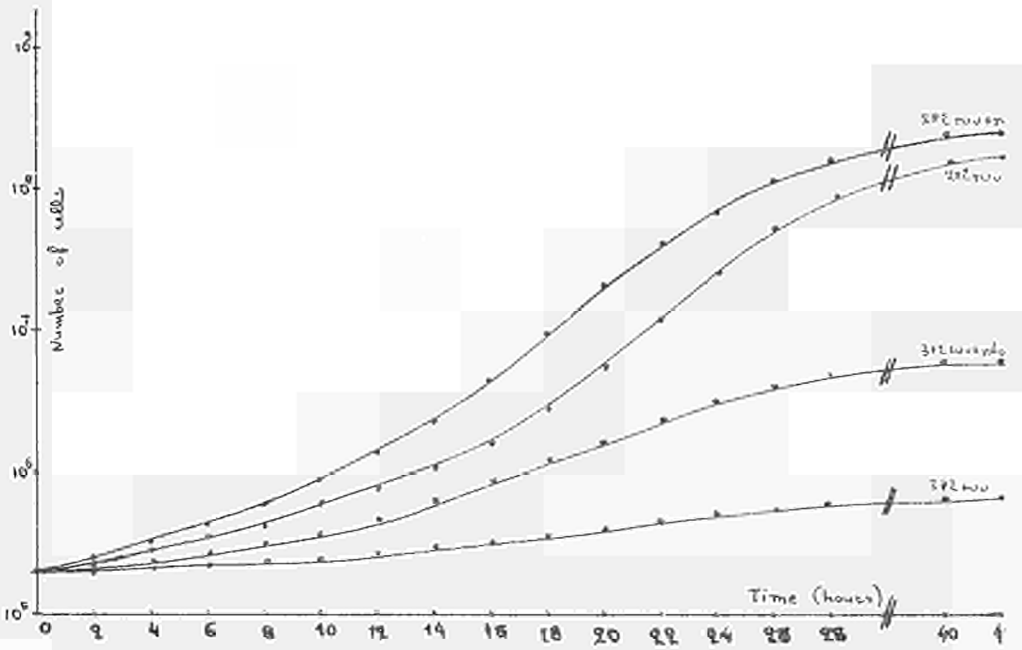
Initial experiments incorporating caffeine, an inhibitor of post replication repair, show an increased killing effect of the mutagen UV when caffeine is present in the solid medium. Experiments with caffeine in liquid medium, however, have indicated that caffeine may be harmful to these particular cells and that is being investigated prior to initiating degradation experiments incorporating caffeine.

Results of Project No. 12.

Head of Project and Scientific Staff: Stelios Piperakis and Dr. E. M. Par

Title of Project: Macromolecular synthesis in ts rad mutant of yeast after radiation treatment.

The rates of macromolecular synthesis and cell multiplication have been studied both before and after mutagen treatment at permissive (28°C) and restictive (37°C) temperatures using the mutant ts rad 109. After UV light exposure (132 J/M^2) and photoreactivating light exposure the cultures show wild type increases in cell numbers, DNA, RNA and protein synthesis at 28°C. At 37° UV light exposure results in severe inhib: of macromolecular synthesis and total cell counts only approximately double over a period of 42 hours after UV treatment. However, as shown in Figure 1 a post UV treatment with photoreactivating light results in a partial suppresion of the UV induced growth delay at 37°. The results clearly implicate the presence of UV induced pyrimidine dimers in the growth inhibition of ts rad 109 at 37°C.



Estimations of DNA, RNA and protein content per cell during the post UV period indicate a normal transition of the cell content of these

macromolecules from exponential phase values to those of stationary phase. There was no evidence of unbalanced syntheses of any macromoles during the treatment period.

Experiments are now continuing with a number of the ts rad mutants isolated in our laboratory.

Results of Project No.13.

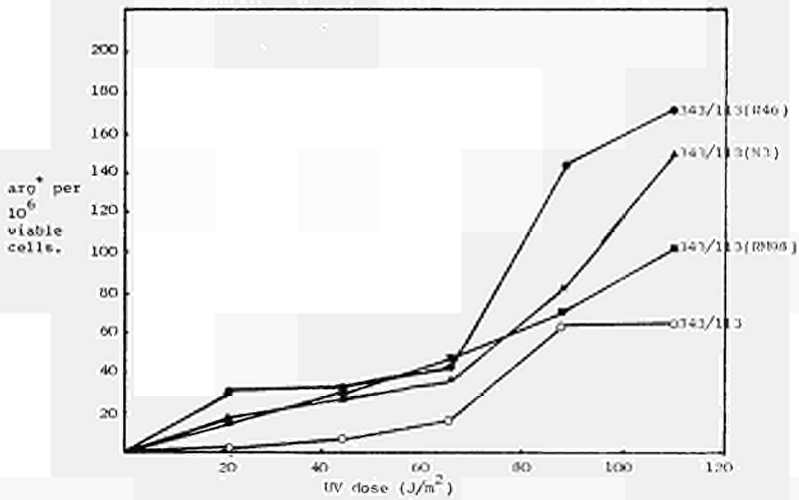
Head of Project and Scientific Staff: Dr. D. J. Tweats

Title of Project: The effect of R(drug-resistance) factors on the detection of mutagens by Escherichia coli K12.

Three R-factors, R46, N3 and RM98, which belong to the N compatibility group, were transferred to Escherichia coli 343/113 gal R^{s}_{18}, arg_{56}, nad_{113}. This E.coli K12 strain has been developed by Mohn et al (Mut.Res.25: 187-19 1974) as a bacterial tester strain for the detection of mutagens.

All three R-factors were found to increase the spontaneous reversion rate at the arg_{56} locus by 2-3 fold. However, the R-factors did not appe to affect the forward mutation rate to gal^{+} from gal R^{s}_{18}. The nad_{113} mutation appeared to be very stable (i.e. probably a deletion mutation) and few revertants were detected in any strain tested.

FIG. 1.



As shown in Fig.1 the three R-factors enhanced the reversion rate at the arg_{56} locus after UV-irradiation. The most sensitive strain 343/11 (R46) was utilised in further tests to determine whether R46 improves the effectiveness of E.coli 343/113 in the detection of chemical mutagens.

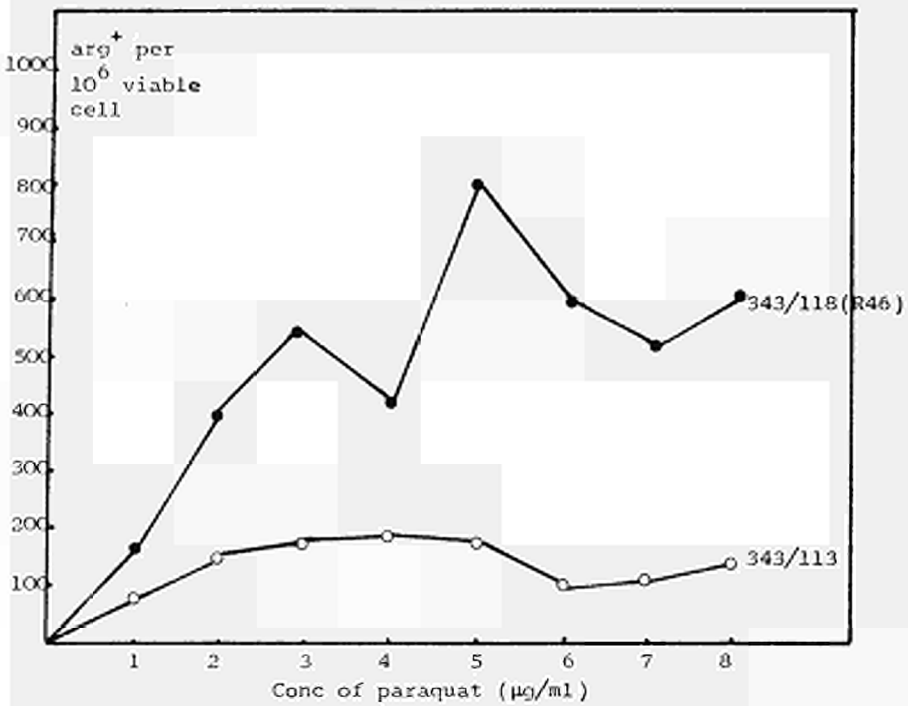
A number of mutagens and potential mutagens were compared for their ability to cause reversion at the arg_{56} locus of E.coli 343/113 and 343/113(R46) by employing conventional spot and liquid tests, with and without an activated microsomal fraction of mouse liver homogenates.

In spot tests the presence of R46 increased the mutational response of the $\underline{arg}_{56}$ locus to alkylating agents and 2-acetyl amino fluorine, but not to cyclophosphamide.

Liquid tests:

The herbicide paraquat has been reported to induce mitotic gene conversion in Saccharomyces cerevisiae (Parry, Mut.Res.21: 83-91, 1973). Attempts to demonstrate mutagenic activity of paraquat in the two E.coli tester strains by spot tests in our laboratory have failed. However, when log phase cells of E.coli 343/113, 343/113(R46) were shaken with paraquat in saline for 18 hours at 37°C, an increase in the number of $\underline{arg}^+$ revertants was detected (Fig.2), and once again mutagenesis was enhanced by R46.

FIG.2.



These results indicate that R46 increases mutagenesis with certain mutagens, but not others. However, on the whole this R-factor increases the effectiveness of E.coli 343/113 in the detection of mutagens.

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Contract N. 111-72-1 BIOI
Prof. G.E.Magni and Prof. N.Loprieno

MOLECULAR NATURE OF POINT MUTATIONS INDUCED BY X-RADIATIONS

Summary - The objective of the proposed research was to obtain in eucaryotic organisms evidence on a large scale on the distribution of molecular types of point mutations in relation to the effectiveness of repair mechanisms. In Saccharomyces cerevisiae the investigation of dose/effect curves for the induction of transitions and transversions at the codon tyr7-1 was concluded. A minor variation with dose of the relative proportions of the two types of base substitution was observed.
Sensitive (lack of repair) and resistant cells do not show any difference for the induction of base substitutions.
In Schizosaccharomyces pombe extensive analysis have been developed for assessing the specificity of the forward mutation induction in different cell cycle stage: G1 and G2 cells have been evaluated and lower doses of X-radiations were more efficient in the production of forward mutation in the cells treated when in G1 stage; at higher doses the same survival-mutation effect relationships were observed independently from the cell stage. The present data make possible the hypothesis of different repair mechanisms operating in the two different DNA structures. Delayed mutations or segregational mutations are at present being evaluated.
The comparison between mutations and gene-conversions induced in the wild type strain (double heterozygotic diploid strain) by X-radiations and the chemical methylmethanesulfonate has allowed the quantitative estimation of the two different genetic effects: whereas gene-conversions were induced with about a frequency of the same degree, MMS was more efficient in the production of gene-mutations.

Project N.1

Prof.G.E.Magni, Dr. S. Sora, Dr. L. Panzeri

Molecular nature of point mutation induced by X-radiation in Saccharomyces cerevisiae

The recent advancements of researches concerning the relationships between mutagenic specificity of X radiations and repair mechanisms have not yet completely solved some basic problems, such as: wich is the molecular specificity of point mutations induced by X-radiations; wether there exists any variation of specificity with the irradiation dose; wether specificity is dependent upon repair mechanisms.
Evidence was recently provided (Lawrence and coll. 1974) that some "rad" mutants blocked in one of the steps of different repair pathways show at the same time an increased sensitivity to the killing action of X-radiations and a decreased sensitivity to the induction of point mutations by the same agent. These facts suggested that repair of point mutations is of "error prone" type.
As far this latter problem rather than compairing wild type strains and rad mutants, we have preferred to use wild type strains only but in physiological conditions where repair mechanisms are fully expressed or repressed. Such two conditions were obtained by isolation of uniformally sized non budding cells from haploid cultures by means of zonal centrifugations. These cells show a uniform high degree of sensitivity to the killing action of X-radiations (Fig. 1). From the above cells, through a synchronized cell division cycle, a uniform population of highly resistant cells was also isolated (see for details Magni et al. Report 1973). Such uniform populations were tested for the induction of transitions and transversions in one specific codon, tyr 7-1, (Magni and coll.1975) with a full range of doses from 2.5 to 20 Krad for the sensitive fraction and from 2.5 to 60 Krad for the resistant fraction according to a procedure already reported (Magni and coll. 1974, 1975).
Our experiments are reported in fig. 2 and fig. 3 and allow some final conclusions:

-- Dose/effect curves show good linearity for the entire range of doses.
-- The relative frequencies of transitions vs transversions are about 25% and 75% respectively at low doses, while at doses higher than 20 Krad the proportions seem to change to 10% and 90% respectively, showing a possible variation of specificity with the dose.

Fig. 1

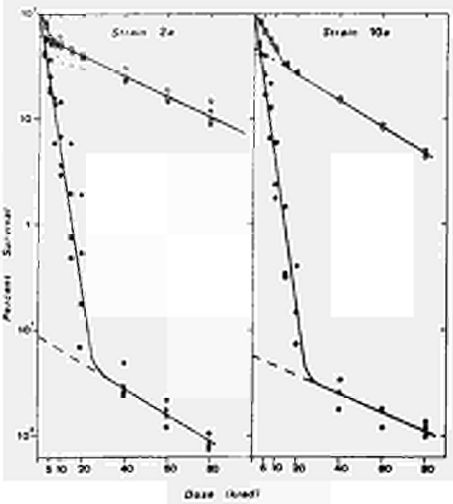

Fig. 2

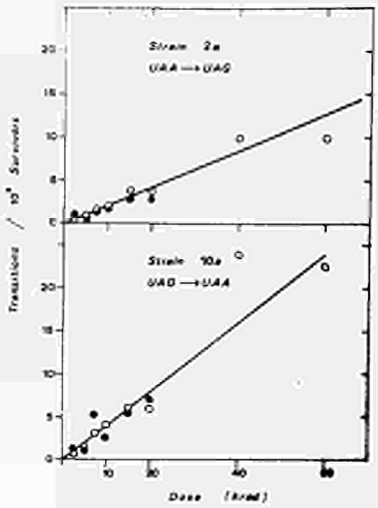

Fig. 3

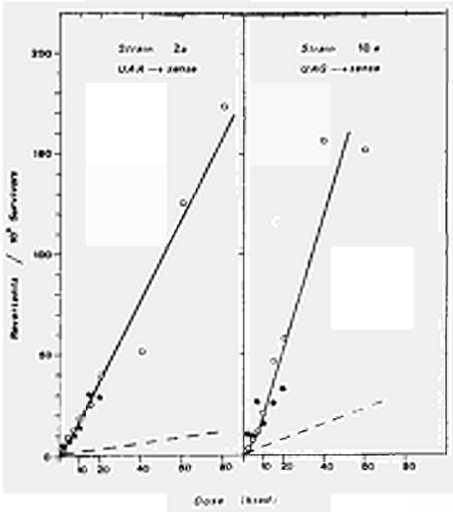

Fig.1 : Survival curves of sensitive —●— cells and resistant cells —○— of strains 2a and 10a.
Fig.2 : Induction of transitions AT⇌GC in sensitive —●— and resistant —○— cells.
Fig.3 : Induction of nonsense→sense mutations in sensitive —●— and resistant —○— cells.

-- No differences were observed between the "sensitive" and the "resistant" cell population. This result is at variance with our preliminary observations (Magni and coll.1974) and with those reported for sensitive cells carrying a rad mutation (Lawrence and coll. 1974). Our data indicate that cells in G1 stage (our "sensitive" fraction) do not posses, in an expression form, some repair mechanisms which have no effect on point mutations of the base substitution type (replication repair or repair of chromosomal aberrations and breakages).

Publications

S.Sora e L. Panzeri - Specificità mutagena dei raggi X e fenomeni di riparazione in Saccharomyces cerevisiae. Atti A.G.I. 1975 in press

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Lawrence C.W., J.W. Stewart, F. Sherman, E. Christensen. 1974 . J. Mol. Biol. 85: 137-162.

Magni G.E., G.P.Sironi, S.Sora, L.Panzeri. Euratom Annual Report 1973 pp. 274-275.

Magni G.E., G.P.Sironi, S.Sora, L.Panzeri. Euratom Annual Report 1974 pp. 304-305.

Magni G.E., C.Frova, L.Panzeri, S.Sora. 1975 Ateneo Parmense acta nat., 11: 181-194.

Project N.2

Prof.N.Loprieno, Dr.S.Baroncelli, Dr.R.Barale, Dr.A.M.Rossi

Molecular nature of point mutations induced by X-radiations in Schizosaccharomyces pombe.

In order to provide a critical analysis of the data presented by Abrahamson et al. (Nature, 245,460-462, 1973) which show a DNA content dependence of the mutation rate of different organisms from bacteria to higher plants, the kinetics of the forward mutation frequency induced by X-radiations in different cell stages of the yeast S. pombe has been analyzed, on treating phosphate sinchronized cells in G1 and G2 stage. The main reasons of such experiments resulted from recent findings of cell stage dependance of repair processes operating in this yeast. We previously have shown (Abbondandolo and Rainaldi,Mutation Res., 27, 235-240, 1975) that G1 cells of an auxotrophic mutant (ade$^-$) were susceptible to the induction of mutation in absence of DNA synthesis and that the mutation frequency was increased three times if the treated cells were allowed to have a DNA replication cycle, but not a cell division. The system we have used in 1975 experiments differs from the previous one, as G1 cells are able to perform DNA synthesis after the treatment and the mutations we are scoring are the results either of direct alterations of DNA base-sequences by X-radiations, or of different repair processes operating during replication or after the replication stage.
G1 cells have resulted in a more sensitivity to the lethal action of X-radiation, the regression coefficient of G2 cells being the double of the G1 cells. It has been observed a more pronounced mutability of G1 cells, compared to the G2: this resulted either from the analysis of the dose-mutation frequency relationship, or from the analysis of survival-mutation frequency relationship.
The higher mutability was particularly evident at lower doses (up to 20,000 Rad : survival 40-60%); the corresponding specific mutation rate values per locus per rad were the following:

G1 = 0.19×10^{-7} x locus x rad
G2 = 0.04×10^{-7} x locus x rad (0.02 when the DNA content is taken in account).

It can be easily seen the influence of the cell stage on the mutability induced by X-radiations in this yeast.
On the basis of the present data and those previously published we may postulate that in S.pombe the main mechanisms of repair involved in the mutational process are represented by a

postreplicationrepair, which is error-prone and requires DNA synthesis. This is expressed when G1 cells are treated and they are allowed to do a DNA synthesis cycle.
A second mechanism is represented by a recombination-repair, which is operating in G2 cells and corresponds to true recombinational events occurring between homologous half-cromatides kept close together by the centromere: this mechanism is more accurate than the previous and leads to higher survival and lower mutation frequency.
The second problem on which we have been interested has been represenred by the study of the kinetics of gene-conversion induction in S.pombe under the influence of two factors: namely (1) the distance between the alleles in a locus; and (2) the nature of initial lesions produced on the DNA molecule.
In our case we have considered three pairs of alleles in the ade7 locus of S.pombe and the two agents X-rays and methylmethanesulfonate (MMS), because these two agents are of a different mutational power in the induction of forward mutations: in the wild type X-rays produce 1.42×10^{-9} mutants per rad per locus, whereas MMS produces 1.32×10^{-5} mutants per mM per min.
The data collected during 1975 show that whereas mutations are induced with a different degree by the two mutagens (10,000 times fold increase in the MMS treatment), the gene conversion frequencies by the two agents differ only by a factor of 10 or less.
For the two agents a linear dose-effect relationship has been obtained, but MMS at higher doses produces gene conversion with a more complex mechanism. The doses under analysis were arranged in such a way to have similar inactivation curves.

The following papers have been published or presented during symposia.

1) Loprieno N. et al.: Mutations induced by X-radiation in the yeast Schizosaccharomyces pombe.Mutation Res. 28,163-173,1975

2) Abbondandolo A. et al.: Radiation-induced mutagenesis and mechanisms of repair in the yeast Schizosaccharomyces pombe. Radiation and cellular control process, Giessen, Germany, Oct. 6-11, 1975.

3) Barale R. et al.: Meccanismi dell'azione mutagena dei raggiX nei lieviti. XXI Annual Meeting of the Associazione Genetica Italiana, Brescia, sept. 1975.

Contractant de la Commission : FONDATION CURIE-INSTITUT DU RADIUM
N° du contrat : 126-74-7 BIOF
Chef des groupes de recherche : R. LATARJET, Directeur
Thème général du contrat : Influences des structures particulières des acides nucléiques sur la nature de leur radio-lésions. Conséquences de l'efficacité des processus de réparation et sur le problème de la radioprotection.

La radiorésistance exceptionnelle de Micrococcus radiodurans est sans doute liée à l'association particulière de l'ADN de cette bactérie avec la membrane plasmique. Le premier projet a permis de mieux définir à l'aide d'inhibiteurs spécifiques et de techniques biochimiques appropriées, la nature de cette association.

Le second projet concernant les cellules eucaryotes (levures et cellules de mammifères en culture) s'est développé cette année dans deux directions majeures. Les relations nucléo-mitochondriales pour les processus de réparation des radiolésions sont comme on le verra plus loin de mieux en mieux définies pour un eucaryote inférieur. La réparation du DNA mitochondrial semble mettre en oeuvre principalement un processus particulier de recombinaison en grande partie gouverné par le noyau. Ces recherches sont à présent étendues à d'autres lésions des ADN, induites par des réactions photochimiques définies ou par un agent chimique.

L'un des objectifs de ces travaux est d'étayer la notion de rad-équivalent chimique dont la nécessité se fait de plus en plus sentir en matière de protection contre les pollutions mutagènes.

La restauration de la survie et de marqueurs génétiques définis par la technique d'hybridation intra et inter espèces de partenaires irradiés ou non a également fait l'objet cette année d'études quantitatives précises.

Enfin, le troisième projet s'attache à définir une radiolésion importante qui a reçu jusqu'ici relativement peu d'attention. Il s'agit du pontage entre acides nucléiques et protéines induit par l'irradiation γ.

Results of project n° 1

Head of project and scientific collaborators : Dr. N. REBEYROTTE et M. DARDALHON-SAMSONOFF

Title of project : DNA-membrane associations and repair in Micrococcus radiodurans after X irradiation.

In 1975, the work on DNA-membrane complexes and repair of X-ray induced damage in Micrococcus radiodurans was followed up. As reported earlier a DNA-membrane complex could be isolated in neutral sucrose gradients after lysis of unirradiated bacteria. In irradiated bacteria (2 x 10^5 R) this complex is dissociated. Reassociation can be observed when the irradiated cells are incubated in complete growth medium (TGY) before lysis.

The DNA-membrane complex was now further characterized. Experiments using different types of neutral sucrose gradients, labels for the DNA and the components of the membrane showed that the complex consists of an association of the DNA with membrane components. Using desoxyribonuclease, ribonuclease, pronase, and phospholipase C, it was demonstrated that the complex formation depends on membrane proteins and lipids as well as on the DNA. RNA does not seem to be involved.

Two new radiosensitive mutants of Micrococcus radiodurans were isolated after treatment with 500 µg/ml NNMG. The DNA-membrane complex isolated from unirradiated and irradiated mutants showed the same characteristics as that of wild type cells. Also the same dissociation and reassociation phenomena exist after irradiation (2 x 10^5 R). Thus, the radiosensitivity of the mutants (factor 2) does not seem to be related to the DNA-membrane complex.

The importance of an unaltered membrane for the dissociation reassociation of the complex is seen in experiments using protoplasts of Micrococcus radiodurans. Less DNA is liberated from the complex after X-irradiation and lysis of protoplasts than after X-irradiation and lysis of complete bacteria. In contrast to the results obtained with complete bacteria no reassociation of the DNA to the membrane takes place, even not after prolonged post-irradiation incubation, when the irradiated protoplasts (2 x 10^6 R X-rays) are incubated in complete growth medium. The absence of the reassociation phenomenon in protoplasts is probably due to

an altered composition of the protoplasts as compared to complete bacteria.

When the irradiated protoplasts are incubated in a medium supporting DNA replication in vitro 20 % of the DNA is able to associate to the membrane during the first hour of post irradiation incubation. It seems possible that the reassociation is related to the onset of DNA replication.

To characterize the reassociation process, we used phenethylalcohol (PEA). This drug inhibits new rounds of DNA synthesis but not RNA and protein synthesis. The cell membrane is thought to be a primary site of the drugs action. The reassociation of DNA to the membrane during post-incubation of irradiated bacteria is below concentrations of 0.25 % PEA partially, above 0.5 % PEA completely inhibited. The effects of PEA may be explained by changes induced in the membrane which inhibit the reassociation. It is possible that the effects of PEA on the membrane and on the initiation of DNA replication are related. However, at the moment it cannot be excluded that it acts parallel on DNA replication and on the reassociation of the complex.

Previously we have shown that the radiosensitizer iodoacetamide acting preferentially on membranes inhibits the reassociation in post incubated bacteria. The inhibitor of protein synthesis, chloramphenicol inhibits only partially the reassociation even in conditions in which the subsequent repair of double strand breaks in DNA is blocked. These results indicate the importance of membrane and certain proteins in the reassociation process. Damage induced in membranes seems to inhibit the reassociation. This failure of reassociation might be the cause for a complete inhibition of a subsequent repair of radiation induced double strand breaks in DNA. So far the most important outcome of this study is the idea that the reassociation DNA-membrane may constitute an important step which allows subsequent repair of radiation damage.

Publication

M. DARDALHON-SAMSONOFF et N. REBEYROTTE. Rôle de l'attachement du DNA à la membrane dans la réparation des radiolésions chez Micrococcus radiodurans. Int. J. Radiat. Biol., (1975) 27, 157-169.

Results of project n° 2 (1)

Head of project and scientific collaborators : Drs. E. MOUSTACCHI, D. AVERBECK, R. CHANET, M. HEUDE, S. HIXON, H. HOTTINGUER-de MARGERIE et N. SCHWENCKE

Title of project : Repair of lesions induced in yeast by radiations and certain chemical agents.

Repair of induced mitochondrial damages.

In UV irradiated yeast cells nuclear DNA demonstrates a very limited degradation and an efficient excision of pyrimidine dimers. In contrast, at the same UV fluences, mitochondrial DNA undergoes an extensive degradation and a retention of dimers is found in the remaining yet undegraded fraction. This result obtained with stationary phase cells explains the enhancement in cytoplasmic "petite" mutants observed after dark liquid holding. For exponentially growing cells after such a post-irradiation treatment a recovery of the wild type genotype is observed. Moreover, in these conditions, we have recently demonstrate a rescue of several mitochondrial genetic markers among the remaining "petite" population. Taken together with the genetic data obtained on mutants specifically UV sensitive to mitochondrial damages (uvsρ), these observations argue in favor of the existence of a repair process for mitochondrial DNA (mit.DNA) in growing cells. Since we show that an excision-repair for mit.DNA does not seem to exist also for log phase cells and even for very low UV doses (L. Prakash, J. Mol. Biol., 1975, 98, 781) it is likely that an error-proone repair mechanism is active in yeast mitochondria. The fate of mit.DNA molecules in UV irradiated growing cells has been followed by the examination of both the kinetics of degradation and the resynthesis by double labelling techniques in CsCl gradients. Preliminary results are in favor of such a reassembling of molecules leading to a recovery of the wild type phenotype.

The events being set into motion after irradiation by γ-rays appear to be quite different in nature.

Biological effects and repair of damage photoinduced by psoralen derivatives.

Denaturation-reassociation studies of yeast DNA treated with the

linear psoralen derivative 3 Carbethoxypsoralen (3 CPs) plus 365 nm light demonstrate that in presence of 3 CPs no cross-links in DNA are formed in the dose range up to 37.8 x 10^4 ergs/mm^2 of 365 nm light. For structural reasons probably only monoadducts are formed involving the 4'5' double bond of the molecule.

Wild type cells of Saccharomyces cerevisiae are more resistant (by a factor of 6) to the photoreactions induced by 3 CPs (monofunctional) than to the lesions induced by 8 Methoxypsoralen (8 MOP) (bifunctional) plus 365 nm light. In comparison to Angelicin (monofunctional), 3 CPs seems to be the more photoreactive compound.

In contrast to results obtained with 8 MOP a synergistic interaction of the two different repair pathways blocked by the rad_2 and the rad_9 mutation is observed after 3 CPs plus 365 nm light. Dark holding experiments show that the excision repair function which is present in wild type and rad_{9-4} cells is important for dark recovery.

We demonstrate that nuclear mutation induction differs greatly after treatment with monofunctional (3 CPs and angelicin) as compared to bifunctional furocoumarins (8 MOP and psoralen), the latter being more efficient. Out of the four compounds 3 CPs is the most efficient inducer of the cytoplasmic "petite" mutation. Data on mitochondrial markers inactivation suggest that it might be a promising agent to study the mitochondrial genome.

Genetic effects of formaldehyde (FA) in yeast.

Stationary phase cells are more resistant to killing induced by FA than exponentially growing cells. We show that this compound induces not only intergenic recombination as already known for Drosophila but also intragenic recombination. Using synchronized populations we demonstrate that the pattern of variations in sensitivity differs greatly from that found for ionizing radiations or UV light. Moreover haploid and diploid cells have the same sensitivity to FA. The analysis of the response of several mutants blocked in the repair of radiation induced damages shows that the excision repair system plays an important role in repairing a fraction of FA induced lesions. The datas allow to calculate a rad-equivalent for this chemical widely used in the industry.

Publications

- Protein synthesis and the recovery of both survival and cytoplasmic "petite" mutation in UV treated yeast cells. I. Nuclear directed protein synthesis. M. HEUDE, R. CHANET et E. MOUSTACCHI. Mutation Res (1975) 28, 37-45.
- Protein synthesis and the recovery of both survival and cytoplasmic "petite" mutation in UV treated yeast cells. II. Mitochondrial protein synthesis. M. HEUDE et R. CHANET. Mutation Res. (1975) 28, 47-55.
- The dose-dependence of the excision of UV-induced pyrimidine dimers from the nuclear DNA's of haploid and diploid Saccharomyces cerevisiae. R. WATERS et E. MOUSTACCHI. J. Bacteriol. (1975) 121, 901-906.
- The fate of UV-induced pyrimidine dimers in the nuclear and mitochondiral DNA's of Saccharomyces cerevisiae on various post-irradiation treatments and its influence on survival and cytoplasmic "petite" induction. R. WATERS et E. MOUSTACCHI. In : "Molecular Mechanisms for Repair of DNA", ed. by P.C. Hanawalt and R.B. Setlow, Plenum Press, New York (1975) Part B, pp. 557-566.
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- Irradiation aux ultraviolets de Saccharomyces cerevisiae : variations au cours de la méiose de la survie et de l'induction de la mutation cytoplasmique "petite" colonie. H. HOTTINGUER-deMARGERIE et E. MOUSTACCHI. C.R. Acad. Sci. Paris (1975) 280, 2617-2620.
- Excision of pyrimidine dimers from the nuclear DNA of a haploid respiratory-deficient (ρ^-) strain of Saccharomyces cerevisiae. R. WATERS et E. MOUSTACCHI. Photochem. Photobiol. (1975) 21, 441-444.
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- Genetic effects of formaldehyde in yeast. I. Influence of the growth stages on killing and recombination. R. CHANET, C. IZARD et E. MOUSTACCHI. Mutation Res. (1975) 33, 179-186.

Results of project n° 2 (2)

Head of project and scientific collaborators : Dr. P. JULLIEN, Mme D. BORNECQUE, D. LAWRENCE, D. SZAFARZ.

Title of project : Effect of X-rays on the ability of mammalian cells to form viable hybrids.

X-irradiation depresses the ability of cells to form hybrids with an unirradiated partner to a lesser extent than their ability to divide and form colonies. The difference between the radiosensitivity of both functions (capacity to form colonies and capacity to form hybrids) is sufficient for obtaining still hybrids with cell populations which have been exposed to X-ray doses sufficient to suppress colony formation.

Such a phenomenon exists as well in intraspecific crosses (mouse cells-mouse cells) as interspecific crosses (hamster cells-mouse cells), cell fusion being induced by Sendai virus. The radiosensitivity of the ability to form hybrids is often biphasic, and the curves are broken. There is no correlation between the radiosensitivity of the colony forming ability of a cell line and the radiosensivity of its ability to form viable hybrids with nonirradiated partners. The nature of the unirradiated partner appears also of little importance. On the contrary the time elapsed between X-ray exposure and virus induced fusion influences the decline of hybrid frequency. In the case of fusion immediately after X-ray exposure, a dose of 5000 R does not suppress the formation of hybrids between 7.5×10^5 irradiated cells and 7.5×10^5 non irradiated cells ; when the fusion is delayed of 24 hours, no hybrids are formed for doses higher than 4000 R. These results were obtained with mouse cells Ag (HGPRT-) crossed with mouse cells Cl 1D (TK-) or hamster cells B1 (TK-), the selection of hybrids depending on the growth in HAT medium. The use as irradiated partners of hamster cell lines without enzymatic defects, BHK21 and RS2-3 (BHK21 cells transformed by Rous sarcoma virus) confirms the possibility to obtain hybrids with cells exposed to relatively high X ray doses (4000 R).

In clones derived from hybrid between irradiated and non irradiated partners, phenotypic markers of the irradiated parent are present : such as chromosomes and species specific antigens, indicating that at least a part of the irradiated genome is replicated and expressed.

When both partners are irradiated before fusion, the hybrid frequency reduction is close to the reduction computed from data obtained after irradiation of each partner alone. For instance, the fusion of 7.5 x 10^5 B1 cells with 7.5 x 10^5 A9 cells induces about 700 hybrids ; irradiation with 1000 R of A9 cells reduces the number of hybrids to 35 (0.035) and irradiation of B1 cells 100 (0.15) ; after exposure of A9 and B1 cells, the number of hybrid colonies is about 4 or 5. Such a correspondence is observed in a dose range of 250 up to 1500 R, in inter and intraspecific crosses and when cell fusion is induced immediately or 24 hours after X-irradiation. But if one considers that in the above exemple, 1000 R reduce the colony forming ability of each parent by about 0.0025, our results allow two ways of interpretation : either it exists a mutual rescue between irradiated cells, either hybrids are formed by cells of which the colony forming ability is less radiosensitive than that of the average population.

Publications

- Viable hybrids between lethally X-irradiated hamster cells and non-irradiated mouse cells. P. JULLIEN et D. LAWRENCE. Submitted to Science.
- A systematic study of the effects of X irradiation on the ability of mammalian cells to form viable hybrids. P. JULLIEN, D. BORNECQUE et D. SZAFARZ. Submitted to Proc. Nat. Acad. Sci. US.

Résultats du projet N° 3

Chef du projet et collaborateurs scientifiques : Dr. B. EKERT et N. GIOCANTI.

Titre du projet : Formation in vitro de liaisons ARN-protéines dans les ribosomes d'E.coli irradiés par les rayons γ.

On sait depuis les travaux de SMITH et ceux de YAMAMOTO que les rayons ultraviolets aussi bien que les rayons ionisants provoquent la formation de liaisons protéines-acides nucléiques in vivo et in vitro. Ces liaisons pourraient être responsables d'effets biologiques importants comme l'inactivation de cellules en culture. Il nous a paru intéressant d'entreprendre une étude in vitro, au niveau des ribosomes, complexé nucléoprotéique naturel dont les activités biologiques sont facilement mesurables.

Nous avons montré que l'irradiation γ de suspensions désaérées de ribosomes 70 s d'E.coli MRE 600, marqués au C 14 uracile, provoque une diminution de l'extractibilité des C14 ARN par le ClLi 4 M - Urée 8 M. En revanche, on observe en contrepartie, l'apparition de radioactivité, proportionnellement à la dose dans la fraction correspondant aux protéines. Cette réaction se produit avec une égale intensité lorsque les ribosomes sont irradiés à l'état dissociés 30 s + 50 s. La présence d'oxygène lors de ces irradiations, inhibe très fortement ce phénomène.

Ces fractions protéiques, issues de ribosomes irradiés à des doses croissantes en absence d'oxygène, furent, soit filtrées sur gels Séphadex G 200, Biogel A 0,5 m, soit centrifugées en gradient de saccharose 5 - 20 % en milieu tamponné en présence d'urée et de ClLi. On déterminait dans chaque fraction le taux de radioactivité et de protéines. Les résultats de ces expériences nous ont amenés à la conclusion que seule une fraction des protéines ribosomales 30 s et 50 s, de l'ordre de 20 % se lie aux ARN ribosomaux sous l'effet du rayonnement. Ces liaisons ARN - protéines s'avèrent relativement stables. Elles résistent à un chauffage de 70° pendant 5 mn. Par contre, elles sont rompues par un traitement alcalin à la soude 0,01 M.

Parallèlement à ces observations, nous avons montré qu'il ne se produit pas de liaisons covalentes entre les ARN 16 s et 23 s, que les ribosomes soient irradiés en présence ou en absence d'oxygène.

Enfin, si on traite par le SDS 0,5 % à 0° des ribosomes diversement irradiés, centrifuge ces derniers sur un gradient de saccharose 5 - 20 %, on isole deux fractions ayant un coefficient de sédimentation proche des ARN 16 s et 23 s et contenant des protéines. Dans ces conditions expérimentales, les altérations radiochimiques éventuelles des ARN (destruction de bases et rupture de chaines polyribonucléotidiques) ne sont pas révélées.

Publication

- Mise en évidence de liaisons ARN—protéines dans les ribosomes d'E.coli irradiés par les rayons γ. B. EKERT et N. GIOCANTI. C.R. Acad. Sci. Paris (1976) sous presse.

Formation of RNA—protein crosslinks in γ irradiated E.coli ribosomes.

γ irradiation, in desaerated conditions of E.coli ribosomes, labelled with C 14 uracil, leads to a decrease in extractibility of C14 RNA by the ClLi 4 M - Urea 8 M. On the other hand, the radioactivity of the protein fraction increases with irradiation. These results strongly suggest that RNA—proteins crosslinks are formed in irradiated ribosomes.

Contractor: National Radiological Protection Board

Contract No.: 131-74-1 BIO UK

Head of research team(s): Dr. G. W. Dolphin

General subject of contract: Radiation-induced chromosome aberrations

In the first project the effect of dose rate and dose fractionation on the yield of chromosome aberrations in human peripheral blood lymphocytes has been examined. In our laboratory it has been found that the best fit of the dicentric chromosome aberration yield (Y) to radiation dose (D) is obtained with the quadratic function $Y = \alpha D + \beta D^2$ where α and β are constants. A physical interpretation of this function is that the linear dose term represents dicentrics produced by a single track and the squared term dicentrics produced by two separate tracks. These two tracks may not be simultaneous and repair mechanisms may intervene and anneal the first lesion so that it cannot react with the second to form a dicentric. Thus by reducing dose rate or fractionating exposures the yield from the βD^2 term will be decreased. The two experiments described in last year's report have now been completed and the results prepared for publication.

Experimental and theoretical studies of the dose effect relationships and the microdosimetry of the irradiation of cultured human lymphocytes have been carried out in Project 2. Some progress has been made in overcoming the technical difficulties of irradiating lymphocytes with α particles and accelerated heavy ions.

Results of Project No. 1

Head of Project and Scientific Staff: Dr. D. C. Lloyd
R. J. Purrott

Title of Project: The effect of dose rate on the yield of radiation-induced chromosome aberrations

Dose fractionation

Blood samples were exposed at 37°C to doses of 500 or 200 rads of 250 kV X-rays at 100 rads per minute. Each dose was split into 2 equal fractions of 250 or 100 rads separated by intervals of 0.25 to 48 hours. The results are shown in Fig. 1. For both doses the yield of dicentrics fell as the time between fractions was increased. The upper reference lines for the 500 and 200 rads single exposures were obtained from X-ray dose response data previously published in reports from this laboratory. Base lines were calculated on the assumption that no interaction takes place between the damage induced by the 2 fractions and are thus twice the yields for the half doses. Fig. 1 shows that by 48 hours the base line of the fractionation effect has not yet been reached for the split 500 rad exposures whilst the data for 200 rads appear to be additive after intervals of only 5 hours. This indicates that lesions are annealed at a rate which may be dose dependent. The first dose fraction may affect the repair mechanism in such a way that a higher first dose leads to slower repair leaving more breaks available for interaction. In the upper graph despite intervals of up to 48 hours, none of the observed dicentric yields or their standard deviation reach the calculated base line. This points to the possible existence of long-term breaks which remain available for recombination long after the majority of damaged sites have been rendered unreactive.

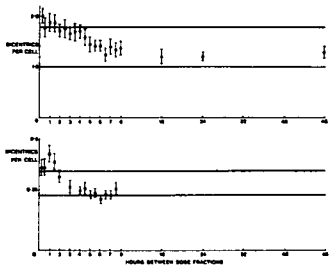

Figure 1: Variation of dicentric yield following X-irradiation with 2 fractions of 100 or 250 rads separated by various time intervals

Dose rate

Doses of 100, 250 and 500 rad from a caesium-137 γ radiation source were given to samples of unstimulated whole blood at 37°C. Dose rates ranged from 1.9 to 400 rads per hour. The results are shown in Fig. 2 in which dicentric data are plotted against dose rate. Only the βD^2 contribution to the dicentric yields are given; the αD component which is not influenced by dose rate has been removed. At the higher dose rates constant yields are obtained and as the rate is reduced a value is reached when a dose rate effect is observed and the yield declines. This effect appears to be dose dependent as the point at which the yields begin to fall varies with dose. This may reflect increased damage to repair mechanisms at the higher doses.

Publications

Purrott, R. J. and Reeder, E. The effect of changes in dose rate on the yield of chromosome aberrations in human lymphocytes exposed to gamma radiation. Mutation Research (in press).

Purrott, R. J. and Reeder, E. Chromosome aberration yields in human lymphocytes induced by fractionated doses of X-radiation. Mutation Research (in press).

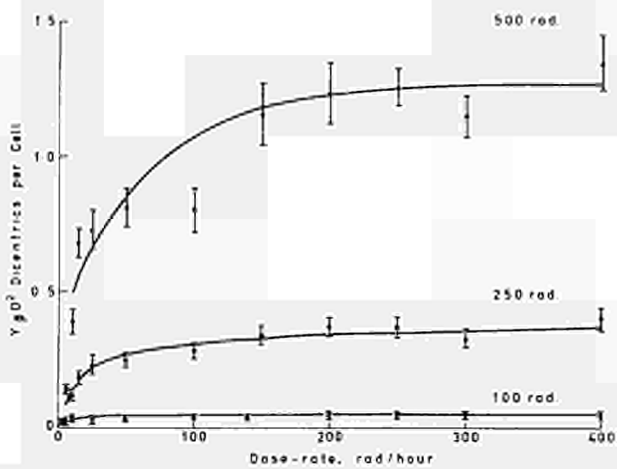

Figure 2: The yield of 2 track dicentric aberrations produced by 3 doses of γ radiation delivered at various dose rates.

Results of Project No. 2

Head of Project and Scientific Staff: J. A. Dennis
B. L. Davies
M. J. Whillock
A. A. Edwards
A. G. Sherwin
R. J. Purrott

Title of Project: The dependence of radiation-induced chromosome aberrations on radiation quality

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A computer program has been written which calculates charged particle spectra and LET spectra when neutrons irradiate materials of biological and dosimetric interest. The pulse height response of a spherical gas-filled counter to neutron irradiation is also calculated. Comparisons between calculated and measured pulse height spectra for counters whose equivalent volume is small have indicated deficiencies in the basic data. In particular the assumed W-values (Dennis, 1973) for heavy ions in tissue equivalent gas have been shown to be in error. An improved formulation of W-value (Dennis and Edwards, 1975) which fits the data of McDonald and Sidenius (1969) just as well as that proposed by Dennis (1971) has resulted in a much improved agreement between the measured and the calculated pulse height spectra for californium-252 irradiation. There remains only one significant discrepancy which would be resolved if the assumed stopping powers of protons in the energy range 70 keV to 1 MeV in in Shonka tissue equivalent plastic were decreased by 10 to 20%. The assumed stopping powers in the plastic were derived by adding the stopping powers of its constituent atoms.

Dose-effect curves for the production of chromosome aberrations in lymphocytes irradiated by various neutron spectra have exhibited an $\alpha D + \beta D^2$ response. Equating the initial slope α to $\frac{1}{D_o}$ where D_o is the dose for one aberration/cell from the intra track term of a dose-effect model, it has now been found that gross sensitive volumes must be at least 3.5 μ in diameter and that the relative biological efficiency of protons of energy greater than 2 MeV is less than 0.1. Attempts to evaluate biological efficiencies of the major neutron produced heavy ions from the neutron dose effect curves have been only partly successful because only 4 neutron spectra have been used.

In an attempt to evaluate biological efficiencies further an apparatus has been designed to irradiate thin blood samples with natural α particles. The problem of culturing the lymphocytes from the sample which is only 35 μ thick has been solved and one preliminary result indicates that the coefficient α is about .01 dicentrics/rad comparable with the response for fission neutrons. The errors in dosimetry were however such that this figure could be in error by as much as a factor 2.

A general theory of dose-effect relationships has been derived based on the following hypotheses:

either (a) biological effects are due to the activation of 2 distinct targets in sites within the cell,
or (b) the effects are due to the co-operation of 2 distinct events in sites within the cell,
and (c) the number of possible sites within the cell is very large.

Hypothesis (a) is that postulated by Neary (1965) and hypothesis (b) by Kellerer and Rossi (1972). These 2 hypotheses lead to essentially similar dose-effect relationships, but different dependence of effect on LET and, in particular, a different prediction for the effectiveness of particle tracks that are shorter than the effective site diameters.

References

Dennis, J.A. Proc. 3rd Symp. on Microdosimetry, Stresa, EUR 4810 d-f-e, 1971.

Dennis, J.A. Phys. Med. Biol., 3, 379, 1973.

Neary, G. J. Int. J. Rad. Biol., 9, 477, 1965.

Kellerer, A.M. and Rossi, H.H. Current Topics in Rad. Res. Quarterly, 8, 85, 1972.

Publications

Edwards, A.A. and Dennis, J.A. The calculation of charged particle fluence and LET spectra for the irradiation of biologically significant material by neutrons. Phys. Med. Biol., 20, 395, 1975.

Dennis, J.A. and Edwards, A.A. Alternative formulations for the energy dependence of W-value. National Radiological Protection Board Memorandum No. 20, 1975.

Edwards, A. A. Charged particle fluence spectra and their relationship to dose-effect curves for neutrons. Proc. 5th Symp. on Microdosimetry, Verbania, September 1975.

Contractor: United Kingdom Atomic Energy Authority
Atomic Energy Research Establishment, Harwell

Contract No.: 134-74-1 BIOUK

Head of research team: D.H. Peirson

General subject of contract: MICRODOSIMETRY STUDIES

The aim of this research is to provide microdosimetric models for the interaction with mammalian cells of radiations of a range of LET's and so improve the basis from which quality factors used in radiological protection are derived. The contract is divided into two projects (a) being the production of biological data from well known cell lines with interpretation in terms of various models and (b) development of new cell lines to test the models on other systems.

Project 1. The mutagenic and lethal effects of neutrons on Chinese hamster cells

A strain of Chinese hamster cells V79-4 has already been established and we have examined the properties of a somatic cell mutation assay which is that of resistance to the purine analogue 8-Azaguanine. This resistance is due to the loss of one of the enzymes involved in the utilisation of purines. Data from the Chinese hamster cells has been compared with that from Tradescantia occidentalis and site diameters have been derived using the theories of Neary[2] and Kellerer & Rossi[1].

Project 2. Development of a mouse lymphoma mutation assay system

The mouse lymphoma L5178Yd has been established in our laboratory and is grown in suspension. The mutation assay system uses a soft agar cloning technique and up to six loci can be examined. The system is now being used to produce mutation and survival data for a range of neutron spectra and γ radiation.

Results of Project No. 1

Head of Project and scientific staff: P.D. Holt
J.C. Asquith
S.J. Boot
J.A.B. Gibson

Title of Project: THE MUTAGENIC AND LETHAL EFFECTS OF NEUTRONS ON CHINESE HAMSTER CELLS

Biological effects of γ radiation

The main effort this year has been on studying gamma radiation effects. As reported last year, we have shown a two-component gamma-induced mutation curve similar to that reported by several authors for both physical and chemical mutagens. It appeared possible that a correlation between the shoulder of the survival curve and the linear component of the mutation curve existed. This has been investigated by altering the shoulder of the survival curve and looking for similar changes in the mutation curve.

Dose fractionation had a marked effect on the rate of mutation induction. The results may be best summarised as showing a linear relationship between induced mutation and survival above a survival level of 20%. This applied to cells irradiated with up to 12 fractions, the maximum number used.

In low dose rate and hypoxic experiments, preliminary results show the same relationship between survival and induced mutation rate as found for dose fractionation. Mutation studies following cell synchronisation by both thymidine and hydroxyurea were unsuccessful. It appeared either that the chemicals themselves act as mutagens or, more probably, blocking DNA synthesis is a mutagenic event.

Plateau phase cells were found to have a very low induced mutation frequency compared to logarithmically growing cells. This is in agreement with the findings of Orkin and Littlefield (1972).[3]

A ^{252}Cf source has been installed. Dosimetry has been carried out and survival curves are now being obtained.

The dual action model as applied to Chinese hamster cells and Tradescantia occidentalis

Neary[2] and Kellerer & Ros[1] have proposed models of radiation damage in eukaryotic cells according to which biological damage results from the interaction of two sub-lesions in a 'site' of diameter d, typically 0.1 - 1 μm. They predict that the dose response curve has linear and square-law terms, corresponding to the production of the two lesions either both by the same particle or by two separate particles:-

$$Y = K(\zeta D + D^2)$$

where Y is the biological yield (for example mutation rate or chromosome aberration yield). D the dose and K and ζ are constants, different for different radiation qualities. In terms of the LET concept

$$\zeta = \frac{72}{\pi} \frac{L}{d^2}$$

where L is the dose mean LET

Hence from an analysis of dose response curves values of ζ and therefore of site diameter d can be found.

For neutron irradiation ζ is large and the square-law term in the dose response curve is not seen. For gamma irradiation ζ is very small and often the response appears to be proportional to D^2. The information which can be obtained from a microdosimetric analysis is then limited.

However, we have shown a linear term in the gamma response of our mutation systems by measuring the response at low gamma doses, and a linear term is also shown in published data on mutation in Tradescantia clone 02 and on chromosome aberration in Tradescantia bracteata. We have analysed these three sets of data.

Since only the linear term can be seen in the neutron response, only the product $K_n \zeta_n$ can be evaluated from this curve. From the two components of the gamma response curve the quantities K_γ and ζ_γ can be obtained, and d can be calculated from ζ_γ since the LET is known. If the gamma response is all due to a dual action mechanism, the value of d obtained from ζ_γ can be accepted and used to calculate a velue of ζ_n, and therefore also K_n, since the product $K_n \zeta_n$ is known. For mutations in Tradescantia clone

02 and chromosome aberration in Tradescantia bracteata the value of K_n found in this way is larger than the value of $K\gamma$, and this is acceptable since reasons have been advanced both by Neary and by Kellerer and Rossi why this should be so.

However, for mutation in Chinese hamster cells K_n found in this way is smaller than $K\gamma$. This seems unlikely to be true, but the conclusion can be avoided if we postulate that part of the linear component of the gamma response is due to a single action mechanism. This would reduce the value of φ_γ for the dual action mechanism, increase d, reduce the value of φ_n and so increase K_n, since the product $K_n \varphi_n$ is fixed by experiment. We have assumed that $K_n = K\gamma$, which gives d = 0.7 μm approximately, but it cannot be excluded that it should be larger (since for both Tradescantia clone 02 and Tradescantia bracteata K_n 2 $K\gamma$). If for mutation in Chinese hamster cells also $K_n = 2\ K\gamma$, the site diameter d would increase to 1.0 μm. The results are summarised in the table.

	Tradescantia clone 02 (mutation)	Tradescantia bracteata (chromosome aberration)	Chinese hamster cells (mutation)
K_n	6.2×10^{-5}	8.3×10^{-6}	7.9×10^{-11}
$K\gamma$	3.7×10^{-5}	4.8×10^{-6}	7.9×10^{-11}
φ_n	406 rads	3360 rads	3420
φ_γ	16 rads	108 rads	533 rads
d	1.6 μm	0.61 μm	0.68 μm
Single Action component	NO	NO	YES

An estimate is available of the sensitivity of Tradescantia clone 02 for cell killing, it is about the same as the sensitivity for mutation, whereas in Chinese hamster cells the sensitivity for mutation is a factor $\sim 10^4$ lower than that for cell killing. The reason for this difference is not clear.

The increased linear component of the gamma response of the Chinese hamster cells, which we have attributed to a single-action type of response, has the effect of reducing the RBE of neutrons at low doses considerably. For example, for a neutron dose of 0.37 rad the RBE is 6.5, whereas

without the 'single-action' response it would be 30. Values of this latter order have been published for both the varieties of Tradescantia mentioned.

Publication

"Application of the dual action model to mutation induction in Chinese hamster cells irradiated with gamma rays and fast neutrons". P.D. Holt. Presented at the Fifth Symposium on Microdosimetry, Verbania Pallanza, September 1975.

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1. Kellerer, A.M., Rossi, H.R.
The theory of dual radiation action, Current Topics Rad. Res. Quarterly 8, 85 (1972).

2. Neary, G.J.
Chromosome aberrations and the theory of RBE. 1. General considerations. Int. J. Rad. Biol. 9, 477 (1965).

3. Orkin, S., Littlefield, J.W.
Mutagenesis to aminopterin resistance in cultured hamster cells Exptl. Cell. Res., 69, 174 (1972).

Results of Project No. 2

Head of Project and scientific staff: J.C. Asquith
P.D. Holt

Title of Project: DEVELOPMENT OF A MOUSE LYMPHOMA MUTATION ASSAY SYSTEM

Survival curves have been obtained for these cells following irradiation by gamma rays and two energies of neutrons (3.5 MeV, 4.5 MeV). The RBE's obtained are in agreement with those previously obtained for Chinese hamster cells.

Toxicity curves for three expressive agents, 6-thioguanine, cytosine arabinoside and thymidine have been obtained. These data are in good agreement with data obtained in another laboratory (Cole and Arlett, submitted to Mutation Research).

This mutation assay system has now been established in this laboratory. Information on radiation induced mutants is being accumulated.

Associato della Commissione:	Università di Pavia
N° del contratto:	112-72-1 BIOI
Capo del gruppo di ricerca:	M. Fraccaro
Tema generale del contratto:	In vitro human gametogenesis (radiation, chromosomal damage in different stages of)

Breve descrizione generale dei lavori compiuti

The work which could be performed with the limited resources at disposal concentrated on the improvement of methods to obtain differentiation of human male germinal cells in vitro. The final goal of this work is to build up a system in vitro to study the effect of radiation and chemicals on human germ cells.

Risultati del progetto N. 1

Capo del progetto e collaboratori scientifici: M. Fraccaro, F. Lo Curto, S. Scappaticci and R. Coco (guest)

Titolo del progetto: Differentiation of male germinal cells in vitro

Descrizione dei risultati

Cell suspensions and tubular fragments derived from a normal human testis were cultured in a standard culture medium after 5h of incubation with 50 Ci/ml of tritiated thymidine. Both sets were cultured up to 25 days. Up to the fifth day numerous cells were present in pachytene, metaphase I and metaphase II, whereas there were relatively few spermatogonia. All of these had an excellent chromosome morphology. By the 10th day no more metaphase II cells were seen and the number of metaphase I cells was decreasing, while pachytenes were still numerous and spermatogonia were increased. After the 20th day only spermatogonial metaphases and metaphases of fibroblasts were seen. The spermatogonial metaphases were easily recognized because of the characteristic morphological appearance of their chromosomes. The cells which were found to be labelled at 5, 10, 15 and 20 days of culture were preleptotene-leptotene, leptotene-zygotene, early and middle pachytene and advanced pachytene, respectively.

We conclude that several cells which were in meiosis when dispersed were capable of maturing in vitro, while spermatogonia and preleptotene spermatocytes did not complete their cycle. Spermatogonia, however, continued to divide mitotically, preserving their chromosome morphology.

Preliminary note published by R. Coco and M. Fraccaro in Clinical Genetics, 8: 395, 1975

Contractant de la Commission : Institut National de la Recherche Agronomique

Contrat Nº 097 - 72 - 1 BIO F

Responsable : Marc A. DALEBROUX, Fonctionnaire Scientifique de la Commission

THEME GENERAL DU CONTRAT :

Etude des effets génétiques, aux plans population et cellulaire, des rayonnements ionisants :

I. Effets des radiations ionisantes sur un caractère de fitness important chez Habrobracon juglandis

II. Réactions génétiques cellulaires aux rayonnements ionisants chez Nicotiana tabacum

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PROJET Nº I

Chef du projet : Marc A. DALEBROUX, Fonctionnaire Scientifique de la Commission

EFFECTS OF IONIZING RADIATIONS ON A FITNESS CHARACTER OF HABROBRACON JUGLANDIS

As mentioned in the last Progress Report, the instability of the Control was investigated by means of a study of the effect of inbreeding. Four inbred lines, A, B, C and D (cf. 1974 Report) were used to build seven experiments which were started when the lines were at the fourth generation of full-sibbing. Experiment Nº 7 was made at the 49th generation of full-sibbing. Seven genotypic classes were compared :

F_0 : consists of a population of 400 random males and 400 random females kept in a population jar every generation.

F_{10}-drift : kept under drift conditions with a sample size of 20 couples. The drift procedure was started after the line had been submitted to full-sibbing for ten generations.

FS : consists of a line that has been long maintained by full-sibbing. It was at its 38th generation of fs for experiment Nº 1, and at its 82^{d} for experiment Nº 7.

A : one of the four inbred lines mentioned above.

(AxB),(CxD) : hybrids obtained by crossing A with B and C with D, respectively.

(AxB)x(CxD) : double hybrid from all four inbred lines.

One experiment consisted of comparing the egg-laying abilities over 25 days, on ten families of six females each, of the seven genotypic classes. The variances (cf. 1974 Report) did not constitute good criteria. Instead, the total number of eggs laid per family was used. The following Teble summarizes the results obtained over the seven experiments through a set of orthogonal comparisons tested in analyses of variances.

Comparison Nº	Genotypic Classes						
	F_0	F_{10}-drift	FS	A	AxB	CxD	(AxB)x(CxD)
1	-3	-3	-3	-3	+4	+4	+4
2	0	0	0	0	-1	-1	+2
3	0	0	0	0	-1	+1	0
4	-1	-1	+1	+1	0	0	0
5	0	0	-1	+1	0	0	0
6	-1	+1	0	0	0	0	0

Comparison Nº	Significance in Experiment Nº						
	1	2	3	4	5	6	7
1	ns	ns	++	++	++	++	++
2	ns	ns	ns	ns	ns	ns	ns
3	ns	ns	ns	ns	ns	ns	ns
4	ns	ns	ns	+	ns	++	++
5	ns	ns	ns	++	++	ns	ns
6	ns	ns	ns	+	ns	ns	ns

ns : not significant ; + : significant at P.05
++ : significant at P.01

From Experiment Nº 3 on, the hybrids as a whole performed much better than the inbred classes. However, there was no significant difference between the double hybrid on one hand and the simple hybrids on the other. Furthermore, among the inbred classes, the jar population and the F_{10}-drift class performed in the long run better than the highly inbred lines A and FS. It is also worth saying that practically no death was observed in the hybrid families, whereas in the inbred classes the deaths became more numerous as the inbreeding increased. It is obvious that hybrids only should be used in subsequent studies on the comparative effects of ionizing radiations and chemical mutagens. A detailed Research Program will be proposed in due time for the next five years together with three other projects on micro-organisms and tobacco.

PROJET Nº 2

Chef du projet : Hubert L. DULIEU, Maître de Recherche au C.N.R.S.
Collaborateur scientifique : Marc A. DALEBROUX, Fonctionnaire Scientifique de la Commission

CELLULAR GENETIC EFFECTS OF IONIZING RADIATIONS IN NICOTIANA TABACUM

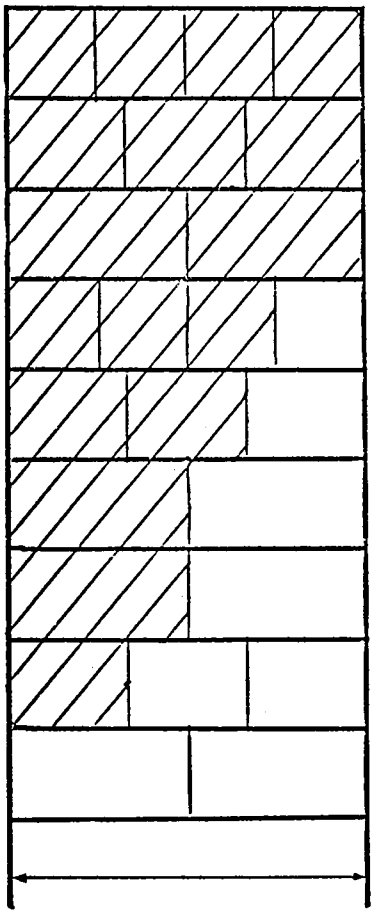
II.1. Variability Components and Functional Interactions at the a_1^+-a_1 and a_2^+-a_2 loci of Nicotiana tabacum

This constitutes further study related to point II.2 of the 1974 Progress Report. The interactions between the two loci were studied in detail through a least squares analysis and can be summarized as follows :

- mutation a_2 is amorphous and probably consists of a small deletion involving gene a_2^+.
- mutation a_1, probably a point mutation, is functional and antagonizes both a_1^+ and a_2^+.
- there is functional co-operation between a_1^+ and a_2^+.

It is hypothesized that the system is ruled by gene dosage compensation, so that, whatever the state of the four loci, a constant amount of a limiting factor utilized by the functional genes modifies the gross action level of the system in terms of the relative proportions of wild and antagonistic genes. The Figure on the next page proposes a schematic model for this gene dosage compensation.

SCHEMATIC MODEL FOR GENE DOSAGE COMPENSATION



4 wild genes, $a_1^+a_1^+\ a_2^+a_2^+$

3 wild genes, $a_1^+a_1^+\ a_2^+a_2$

2 wild genes, $a_1^+a_1^+\ a_2a_2$

3 wild genes and
1 antagonistic gene, $a_1^+a_1\ a_2^+a_2^+$

2 wild genes and
1 antagonistic gene, $a_1^+a_1\ a_2^+a_2$

1 wild gene and
1 antagonistic gene, $a_1^+a_1\ a_2a_2$

2 wild genes and
2 antagonistic genes, $a_1a_1\ a_2^+a_2^+$

1 wild gene and
2 antagonistic genes, $a_1a_1\ a_2^+a_2$

2 antagonistic genes, $a_1a_1\ a_2a_2$

available amount of the limiting factor

: chlorophyll part of the expressed phenotype

: deficient part of the expressed phenotype

II.2. Effect of Acute Low-Doses Gamma Irradiation in Nicotiana tabacum. Dose-Response Relationship. Preliminary Study

This study bore on the $a_1^+a_1$ $a_2^+a_2$ greenish-yellow mutant of tobacco. A previous investigation (cf. 1974 Report) of the dose-response relationship at 0, 8, 16, 32, 64, 128 and 256 R of gamma-rays from a ^{60}Co source showed the necessity of studying the response between 0 and 32 R, with special interest in the 0 - 8 R range. The methods used and the preliminary results obtainad are as follows.

Individuals to be irradiated were transplanted as cuttings from culture tubes into pots and exposed, at a young vegetstive stage (10 - 15 cm), to a ^{60}Co source with an intensity of 350 R/h. The effects were observed in terms of number of reverted (green) areas on leaves that had undergone divisions after the treatment. All observations were made at the 2^d, 3^d and 4^{th} foliar levels above the last leaf that had terminated cell division at the moment of the treatment. The experimental unit was constituted by five plants, and five repetitions were made for each dose-treatment. The data were recorded as the total number of reverted areas for each experimental unit. The doses applied were 0, 1, 2, 4, 8, 16 and 32 R.

Owing to the type of the response, the experiment was split into three overlapping parts : 0-1-2, 2-4-8 and 8-16-32 R. This was done to prevent interference, in the statistical analysis, between apparently different types of response. Also, the splitting was justified by the heterogeneity of the sampling variance from one group of doses to another.

In the 0-1-2 R group, the response was found to be quadratic.

In the 2-4-8 R group, the response detected was statistically horizontal, thus showing a plateau.

In the 8-16-32 R group, the response was found to be linear. However, the foliar levels modified the linearity : there was one equation for the second level, and another for the third and fourth levels together.

Figure 1 presents the adjusted response curves. It seems that the difference between the linear responses in the third group is due to a "dilution" of the response as the foliar level observed gets higher : the number of cells present in the successive primordia at the moment

Figure 1. Adjusted responses for 5-replicate totals (25 plants)
0-1-2 and 2-4-8 R over 3 foliar levels
8-16-32 R: Y_1 over 2nd level
Y_2 over averaged 3rd & 4th levels

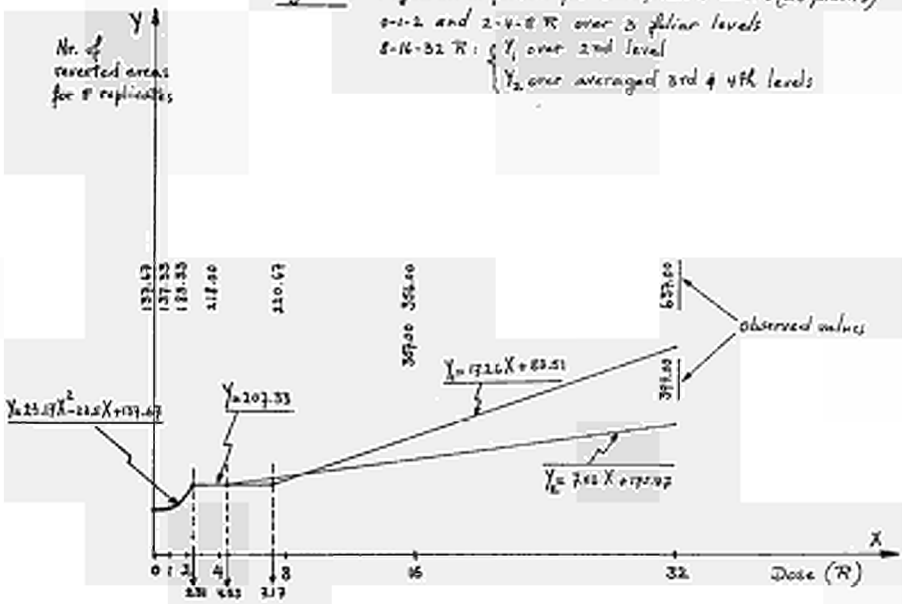
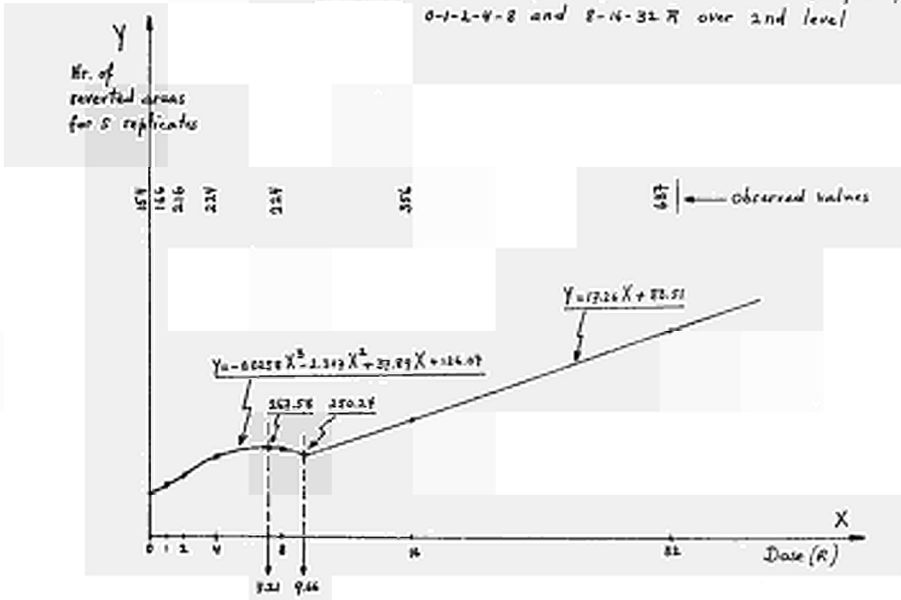


Figure 2. Adjusted responses for 5-replicate totals (25 plants)
0-1-2-4-8 and 8-16-32 R over 2nd level



of the treatment decreases very rapidly, making the sample size insufficient. Therefore, a new analysis was made on the second level only. Furthermore, since the response had plateaued in the 2-4-8 R group, it did not seem unreasonable to put the first two groups together in order to test an eventual cubic response and so check the theoretical presence of a sensitivity peak between 2 and 8 R. The cubic component was at the limit of significance at P.05 in group 0-1-2-4-8 R. Therefore, a 3^{d}-degree response curve was adjusted, keeping in mind that this constitutes only a preliminary investigation and that the 2-8 R range will have to be studied in more detail. Figure 2 presents the adjusted response curves. The gross shape of the adjustments in Figure 2 does not drastically differ from that in Figure 1. There is no convincing evidence of the presence of a response peak between 2 and 8 R : the interpolated maximum response is 267.58 for 7.21 R while the largest adjusted value is 265.64 for 8 R.
Of course, as stated previously, a more thorough investigation of the 2-8 R range will be needed to allow more secure conclusions.

PUBLICATIONS

Dalebroux M. A., 1974. Régressions Polynomiales par l'Origine. Analyse de Variance par la Méthode des Coefficients Orthogonaux. Ann. Amélior. Plantes, 24 (1) : 71 - 76.

Dalebroux M. A. et H. L. Dulieu, 1974. Estimation aux Moindres Carrés des Effets Géniques chez un Organisme Diploïde - Un Locus à Deux Allèles - Application à Nicotiana tabacum. Genetica, 45 : 61 - 70.

Dulieu H. L. and M. A. Dalebroux, 1975. Spontaneous and Induced Reversion Rates in a Double Heterozygous Mutant of Nicotiana tabacum var. Xanthi N.C. - Dose-Response Relationship. Mutation Research, 30 : 63 - 70.

Dulieu H. L. and M. A. Dalebroux, 1975. Effect of Acute Low-Doses Gamma Irradiation in Nicotiana tabacum L. - Dose-Response Relationship. Preliminary Study. European Community , Contact Group Meeting on the Genetic Effects of Radiations. London, November 19-21.

Dulieu H. L. et M. A. Dalebroux (proposé à l'éditeur). Composantes de Variabilité et Interactions Fonctionnelles aux Loci $a_1^+-a_1$ et $a_2^+-a_2$ de Nicotiana tabacum.

Contractor: Carlsberg Laboratory, Department of Physiology

Contract No.: 124-74-1 BIODK

Head of research team(s): Prof. Dr. Diter von Wettstein

General subject of Contract: Mutation Spectra of Eceriferum Genes

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An evaluation of the genetic health hazards of mutagens and their usefulness in plant and animal breeding requires precise information on the genetic variation induced by individual mutagens. We have therefore isolated over the last 16 years 1180 eceriferum mutants in barley, i.e., mutants with an organ specific change of the wax coating and assigned 1065 recessive mutants to 59 gene loci spread across the seven barley chromosomes. The mutagens studied include acute and chronic irradiation with X- and γ-rays, neutrons, ethyleneimine, and sulfonates. Highly significant differences in the mutation spectra are obtained. Whereas X-rays produce a rather unspecific mutation pattern, mutagens like neutrons, ethyleneimine, and sulfonates hit certain genes more than others. The genes that mutate preferentially with the latter three mutagens are quite different ones.

With the aid of translocations, 27 of the 59 genes have so far been assigned to chromosome arms and 10 genes have been located with the aid of three point tests on the genetic map. The location of genes that show specific affinity to certain mutagens is fundamental to further inquiries into the nature of mutagen specificities. The material has been used to calculate forward mutation rates of the eceriferum genes after acute irradiation with neutrons and X- or γ-rays. The mutation rates per rad and genome are comparable to those found with fungi and bacteria. There is thus no indication that genome size has a major influence on the induced mutation rates of individual genes.

The mutants analysed genetically in this project serve as tools in studies on the structural organization, composition and biosynthesis of very long chain lipid molecules (fatty acids, hydrocarbons, diketones, alcohols, esters) in the epicuticular wax which constitutes the border of the plant surface and the surrounding atmosphere.

Results of Project No.: 1

Head of Project and scientific staff: Prof. Dr. D. von Wettstein
Ms. U. Lundqvist
Dr. P. von Wettstein
Stud.scient. J. Dalgård-Michelsen

Title of Project: Mutation spectra of eceriferum genes after treatment with ionizing radiations and chemical mutagens

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The distribution of the 1065 eceriferum mutants among the 59 loci is presented in the following table, in which the loci are arranged according to the number of alleles known.

Locus	c	u	q	i	za	a	zj	j	t	n	ze	b	e	g
Alleles	148	125	122	60	55	41	40	40	40	38	37	32	31	28
Locus	p	x	w	s	zi	zc	zh	zk	d	z	h	r	zd	zn
Alleles	26	20	18	15	12	11	11	10	9	7	5	5	5	5
Locus	zo	zp	yb	v	zb	zs	f	o	zl	zv	ye	yh	zr	zt
Alleles	5	5	5	4	4	4	3	3	3	3	3	2	2	2
Locus	zu	zz	yc	yf	k	l	m	y	zf	zg	zq	zw	zx	zy
Alleles	2	2	2	2	1	1	1	1	1	1	1	1	1	1
Locus	ya	yd	yg									Total		59
Alleles	1	1	1									Total		1065

The mutation spectra and mutagen specificities as reported in 1975 are supported by this extended material.

In 1973 Abrahamson, Bender, Conger and Wolff (Nature 245, 160) proposed that the radiation induced mutation rate is proportional to the total genome size, implying that genes or regulons in animals, plants, and man are larger than in bacteria. To support their proposal they use two very high figures for forward mutation rates in barley. The analysis to be presented below reveals these figures to be in error and that the radiation induced mutation rates of the eceriferum genes in barley are of the same order of magnitude as those determined for bacteria and yeast, i.e., species with very much smaller genome sizes. It should be pointed out that the existence of effective cellular repair mechanisms can be a source for sizable variations in mutation rates within a species if different strains or different stages of the life cycles are compared.

In this material surprisingly uniform mutation rates have been observed: In 20 mutation experiments with acute irradiation using sparsely ionizing radiation carried out between 1949 and 1972, the mutation rate for 59 eceriferum genes determined per rad and spike progeny ranged between 0.8 and 9.4 x 10^{-7}. In 29 experiments employing neutron irradiation carried out between 1952 and 1972, the mutation rate per rad and spike progeny varied between 0.4 and 2.4 x 10^{-5}.

For the total material the following figures are found:

	X-RAYS, γ-RAYS Mutations	Spike progenies x rad				Rate
Total	92	449	353	212		2.05 x 10^{-7}
59 loci	92		- " -		Per locus	3.47 x 10^{-9}
Locus c	12		- " -		- " -	26.71 x 10^{-9}
Locus j	1		- " -		- " -	2.23 x 10^{-9}
	Neutrons					
Total	264	28	002	219		94.28 x 10^{-7}
59 loci	264		- " -		Per locus	1.60 x 10^{-7}
Locus i	44		- " -		- " -	15.70 x 10^{-7}
Locus w	1		- " -		- " -	0.36 x 10^{-7}

Sizable differences are found for mutation rates of individual loci.

If the figures are calculated per haploid genome and corrected for the fact that some of the spike primordia in the irradiated kernels have more than one cell, the following data are obtained:

	MUTATION RATE	
X-RAYS	Per rad, per locus per spike progeny	3.47 x 10^{-9}
	Per haploid genome	1.74 x 10^{-9}
	Per cell (1.5)	1.17 x 10^{-9}
NEUTRONS	Per spike progeny	1.60 x 10^{-7}
	Per haploid genome	0.80 x 10^{-7}
	Per cell (1.5)	0.53 x 10^{-7}
X-RAYS	E.coli	1 x 10^{-9}
	Saccharomyces	6 x 10^{-9}
	Neurospora	3 x 10^{-9}

The following arguments can be presented that the majority of the eceriferum mutations are likely to be intragenic mutations:

a) Eceriferum genes control the synthesis of the molecules contained in the surface waxes, which are counted as secondary plant substances not required for the life of the plant.

b) By working with such genes, one selects automatically against deletions extending outside these genes since intergenic deletions will easily include a vital gene and then not be represented among the mutants studied.

c) As in all grasses studied, deletions cause defects in the functioning of the pollen grains and therefore aberrant segregation ratios, i.e., deficits of recessives are a sensitive test for the occurrence of deletions.

In progenies ranging from 40 to 1036 individuals, we have studied segregation ratios for 16 X-ray induced mutants belonging to 16 different genes. Among 133 progenies analysed 113 revealed normal segregations and these occurred in all 16 mutants. The 26 progenies giving significant deficits of recessives could be ascribed to other markers distorting the segregations in the cross.

Likewise, for six neutron induced mutants 44 of 55 tested progenies gave very good segregations, and of 14 mutants induced with chemical mutagens studied in 121 progenies 98 gave entirely normal segregations. For the 36 studied mutants thus no evidence was obtained that these are the result of chromosome deletions.

The results are being prepared for publication.

Publications

P. von Wettstein-Knowles. Tracking down β-diketone synthesis with the aid of the eceriferum mutants. In Barley Genetics III. Proc. 3rd Internat. Barley Genet. Symp. Garching, Germany, 1975 (in press).

A.G. Netting and P. von Wettstein-Knowles. Biosynthesis of the β-diketones of barley spike epicuticular wax. Arch. Biochem. Biophys. (in press).

P. von Wettstein-Knowles. Biosynthetic Relationships between β-diketones and Esterified Alkan-2-ols Deduced from Epicuticular Wax of Barley Mutants. Molec. Gen. Genetics (in press).

Contractor: The Finsen Institute, Copenhagen

Contract No.: 120-73-1-BIO-DK.

Head of research team: Mogens Faber

General subject of Contract: Radiation Sensitivity of the Human Ovary.

During the year 1975 the work on Radiation Sensitivity of the Human Ovary continued and concerned itself with the following questions:

1. The normal development of the human ovary
2. The development of the ovary after irradiation and cytotoxic drugs
3. Follicular atresia in the human ovary
4. Study of the spacial distribution of follicles in the infant human ovary.

Publications:

Peters, H., Byskov, A.G., Himelstein-Braw, R., Faber, M.: Follicular Growth: the Basic Event in the Mouse and Human Ovary. J. Reprod. Fert. (1975) 45, 559.

Himelstein-Braw, R., Byskov, A.G., Peters, H., Faber, M.: Follicular Atresia in the Infant Human Ovary. J. Reprod. Fert. (1976) 46, 55.

Peters, H., Himelstein-Braw, R., Faber, M.: The Normal Development of the Ovary in Childhood. Acta Endocr. (1976) in press.

Results of Project:

Head of Project and scientific staff: Mogens Faber
Anne Grete Byskov
Hannah Peters
Ruth Himelstein-Braw.

Title of Project: Radiation Sensitivity of the Human Ovary.

1. The normal development of the human ovary.

In continuation of the study a classification of follicles in the human ovary was devised which defined the progressive stages of follicle development. Furthermore different stages of ovarian development were recognized which made it possible to define the state of the organ after certain influences (radiation, disease, drugs etc.).

2. The development of the ovary after irradiation and cytotoxic drugs

Heavy irradiation to the abdomen during childhood severely damages the ovaries by greatly reducing the number of oocytes and preventing follicle growth. Cytotoxic drugs used in the treatment of childhood leukemia inhibit follicle growth in all cases treated for one month or longer.

3. Follicular atresia in the human ovary

Follicle atresia has been defined. It occurs in all stages of follicle development. However, the percentage of follicles with signs of atresia became larger as the size of the follicles increased.

4. Study of the spacial distribution of follicles in the infant human ovary

This study is in progress. In serially sectioned ovaries all follicles were registered in a coordinate system. Several computer programs are used to study the spacial relationship of follicles within the organ in different growth phases.

Contractant: University of Rome, (Istituto di Chimica Biologica)
Contract No. 146-75-1 BIOI
Head of Research group: E.P. Whitehead
General theme of contract: Enzymology of ATP-dependent DNAases involved in repair of radiation damage
Collaborators: Prof. P.M. Fasella,
Dr.ssa F. Riva,
Dr.ssa G. Cerio-Ventura,
Dott. C. Salerno

General Description of work and Results

The purpose of the present project is the investigation of the mechanism of ATP-dependent DNAases known to be involved in the recombinational repair of radiation damage in bacteria. Since this years work has served only to create the necessary technical bases for this investigation it needs only to be recalled here that problems we consider suitable for early attack are: clarification of the stoichiometry and coupling of the ATP hydrolysis catalysed by this type of enzyme with that of DNA hydrolysis; analysis of the presumed "one-by-one" mechanism of attack on large DNA molecules and the question of the attack by the enzyme on its own initial products.

The activity of 1975 has consisted in the initial organisation of the laboratory for this project, the purification of the ATP-dependent DNAases to be studied, and the purification of other nucleases to be used in the analysis of their mechanisms.

As stated in the research proposals it has been decided to work with ATP dependent DNAases from both Micrococcus luteus and Escherichia coli in order to enable comparative stu

dies. The ATP-dependent DNAase from M. luteus has been purified to near-homogeneity and is free of DNA-independent ATPases. The purification procedure worked out here differs from that in the literature, and consists in chromatography on DEAE-Sephadex with elution by phosphate buffer at pH 6.7 which gives increased resolution and avoids problems of instability encountered with other methods earlier used, followed by chromatography on Sephadex G-200 and on hydroxylapatite. It has been found possible to use commercially available micrococcus preparations as a source of the enzyme. A preliminary kinetic characterisation of this enzyme has been carried out. The method used for purification of the E coli enzyme (exonuclease V controlled by the genes rec B, rec C) is that of v. Dorp, Benne & Palitti (1975). So far liberation of the E. coli enzyme from DNA independent nucleases has not been achieved. Preliminary kinetic and mechanistic experiments are however being carried out. The following enzymes required for the analysis of the action of the above two have been purified to a state of freedom from other contaminating nuclease activities: exonuclease I from E. coli and endonuclease from aspergillus.

Reference

B. van Dorp, R.Benne & F.Palitti, (1975) Biochim. Biophys. Acta, 395 446-454.

Contractor:	The Polytechnic of Central London
Contract No.	142 - 74 - 7 BlO UK
Head of research team:	Dr. G. Holt
General subject of Contract:	Studies of gene mutation, mitotic recombination, chromosomal non-disjunction and deletion induced by radiation.

The research, so far, has developed along two main lines. Firstly, a system is now in use for the assessment of radiation damage with respect to gene mutation, mitotic recombination and non-disjunction in the same experiment. The induction of non-disjunction and mutation by radiations with different LET values has been compared. Secondly, work is in progress to examine the frequency of chromosomal deletions, produced as a result of possible single and double events, with change in LET as a first step in applying existing microdosimetric data to the interpretation of biological effects.

Results of Project No. : 1

Head of Project and scientific staff : Dr. I. D. Normansell
Dr. V. Karunakaran

Title of Project : Mutational studies in Aspergillus nidulans

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(A) A green-sporing strain of the eukaryotic organism Aspergillus nidulans was used. Genetic markers on chromosome I of this strain (C471D) are shown below:

fpaB37	galD5	suAladE20	+	riboA1	anA1	pabaA1	yA2	adE20	biA1
+	+	+	sulA1	+	+	+	+	adE20	+

conidial colour markers: y = yellow
auxotrophic requirements: an = aneurin, bi = biotin, ad = adenine
paba = p-aminobenzoic acid, ribo = riboflavin

resistance to antimetabolites: fpa = p-fluorophenylalanine.
sul = sulphonamide

others : gal = inability to use galactose as sole carbon source
suAladE20 = suppressor of adE20

The following radiations with LET values ranging from approximately 0.3-10.0 keV/µm. were chosen for this work:-

low { Electrons (15MeV, dose rate 1 krad sec^{-1})
{ γ - rays (1.25 MeV, dose rate 3 krad min^{-1})
medium - X - rays (50kv, dose rate 282.4 rad min^{-1})
high - β -particles (6 keV, dose rate approx. 1 krad hr^{-1})

Survival curves differed markedly (Fig. 1.) The shallowest curves were given by radiations with low LET values. With increased LET correspondingly greater killing was observed, at least over an initial period. The lowest energy source, soft β - particles posed many problems in calculating dose. The dose delivered by 100 µCi of tritiated water (HTO) at one time throughout the experiment was estimated as 1 krad hr^{-1}. However, as the water inside the cell was in flux with the tritiated water in which the cell was suspended, that dose would not be delivered until all the cell water had been replaced by HTO. In addition a further complication arises since the average track length of β -particles from tritium is 0.8 - 1.0µm in water. This is less than the diameter of the cell (4.2 µm).

Surviving colonies were examined for the presence of yellow sectors. Of the three radiations examined, soft β -particles were found to

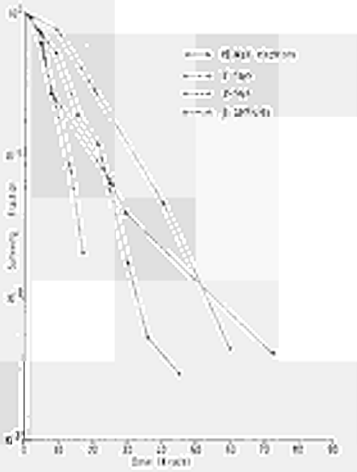


Fig. 1. Survival curves for a diploid strain of A.nidulans (C471D) after treatment with ionizing radiations.

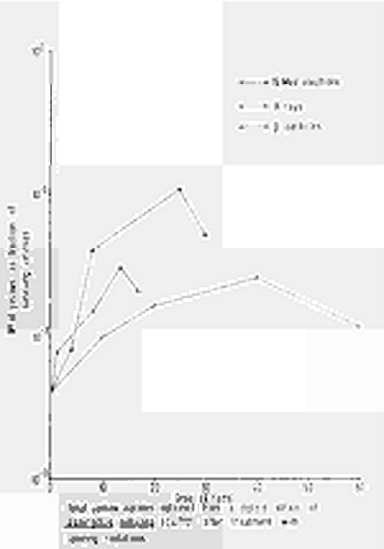


Fig. 2. Total yellow isolates obtained from strain C471D

induce the highest level of genetic damage (Fig. 2.) Yellow sectors could have arisen in one of several ways. 'Yellows' produced as a result of chromosomal non-disjunction, mitotic recombination or gene mutation could be distinguished. Figs. 3 and 4 show that β -particles from HTO induced the highest frequency of non-disjunction and gene mutation. Mitotic recombination was found to occur at an extremely low frequency throughout the experiments and was not affected significantly by changes in LET. In recent work selection has been made for resistance to p - fluorophenylalanine and sulphonamide and non-disjunctional types were distinguished from mitotic recombinants initially by the spore colour of surviving colonies.

(B) The genotype of the strain of A.nidulans employed (GH5) in studies of chromosomal deletions is shown below:

I	proA1	pabaA6	yA2	+	+
II			+	adE20	biA1

markers as for strain C471D above with pro = proline auxotrophy

This strain possesses a portion of chromosone I in duplicate (one in the normal position and one translocated to linkage group II. Survival curves for this strain after treatment with γ - rays and X - rays are presented in Fig. 5. It was possible to recognize among the survivors loss of one or more dominant alleles due to deletion. For example, loss of part of the duplicate segment bearing the dominant allele $yA2^+$ resulted in yellow colonies so that in the same experiment, dose-effect curves were produced showing total survival as well as differences in genetic constitution among the survivors (Fig. 5.) The approximate size of each deletion and the segment involved can be determined genetically. However, problems have been encountered with the low level of deletions induced and studies have concentrated on methods to increase the sensitivity of the system. A number of chemical agents known to increase the radio-sensitivity in other organisms were tried and caffeine has shown promising results. The introduction of a uvs mutation (conferring sensitivity to ultraviolet light) on chromosome IV of a strain carrying the same I/II duplication significantly increased the number of deletions produced.

Publications

Karunakaran, V. and Holt, G. (1976) "Genetic maps and physical units" in Proc. 5th Symp. on Microdosimetry, Verbania 1975.

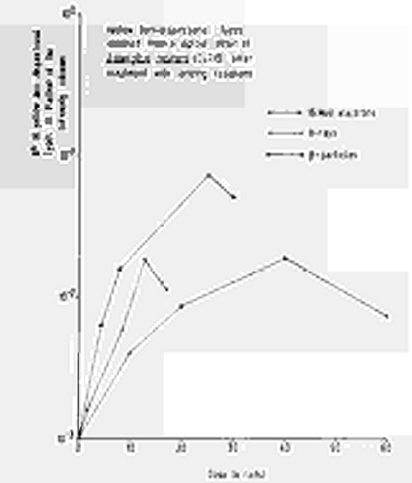


Fig. 3. Yellow non-disjunctional types obtained from strain C471D

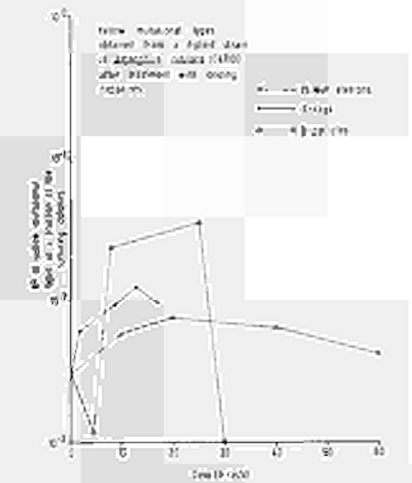
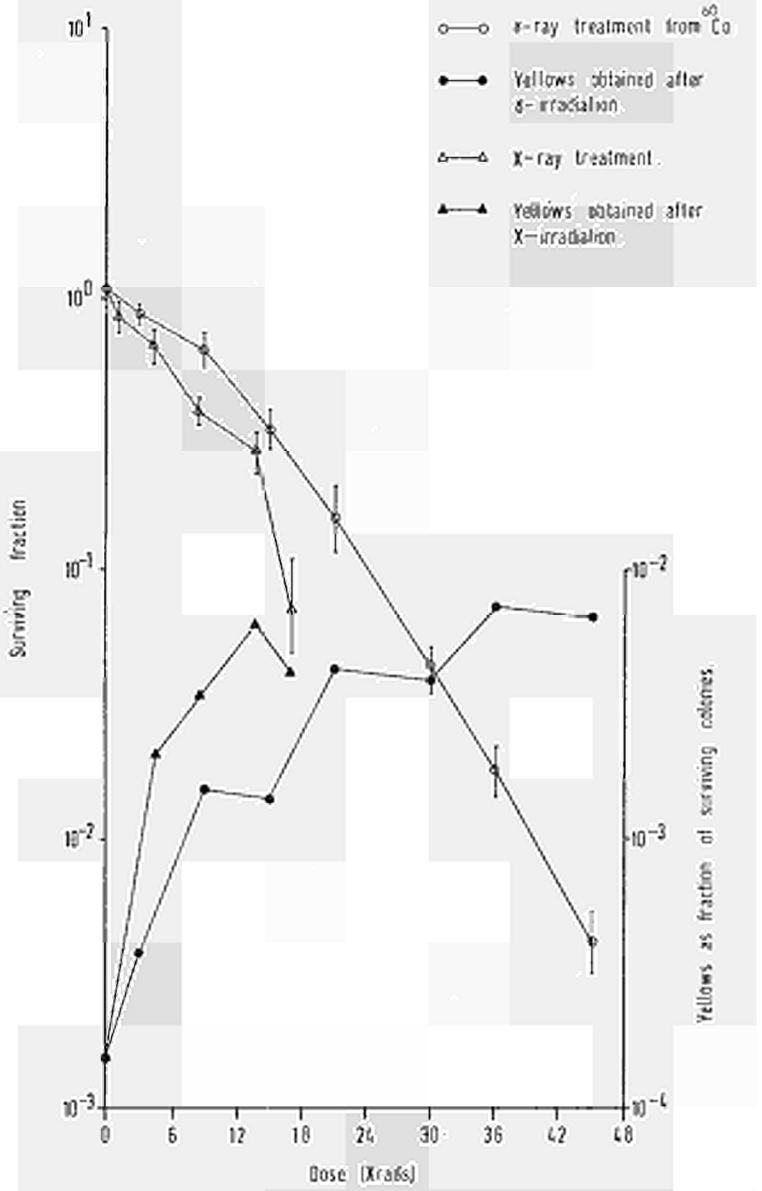


Fig. 4. Yellow mutational types obtained from strain C471D



Survival curves and production of deletions in Aspergillus nidulans strain CH5 by ionizing radiation.

Fig. 5. Survival curves and production of yellow deletion types in strain CH5

Contractor: College of Technology, Kevin Street, Dublin 8, Ireland.

Contract No: 148-75-1 BIO EIR.

Head of Research Team: J.K. Taaffe; J.F. Malone.

General Subject of Contract: THE EFFECT OF NORMAL PHYSIOLOGICAL AGENTS ON THE ABILITY OF CELLS TO REPAIR AND RECOVER FROM RADIATION DAMAGE.

This project aims to establish if some normal metabolic agents influence repair of radiation damage in mammalian cells. Five agents were selected for study because available evidence suggested they might give positive results. The agents were Vitamin A, Hydrocortisone, phytohaemaglutinin, cyclic AMP, and oxygen. An underlying theme of the project is that factors in normal cell physiology, particularly membrane effects, may have an important role to play in repair and recovery. This role is probably complimentary to that of DNA and chromosomes.

If the investigations yield positive results, known effects of the chosen agents will be used to guide the type of investigation of radiation repair to be undertaken. The conclusions may be useful in dealing with victims of acute radiation accidents, and those who receive relatively large radiation doses, through extensive sequential use of diagnostic radiology, or radiotherapy. In the latter case, the fact that the agents are normal metabolites could be of assistance in designing regimes to preserve normal tissue.

The project required that we initially establish if these agents influenced the behaviour of cells in their own right. It was also necessary to devise statistical criteria to guide selection of models on which interpretation of data in the low dose region of survival curves be based. These two areas constituted the main field of investigation during 1975.

Results of Project No. 1.

Head of Project and scientific staff: Dr. J. F. Malone;

Mr. I.A. Kinsella; Ms. M. Delaney; Dr. D. Hickey;

Mr. M. O'Connor.

Title of Project: THE EFFECT OF NORMAL PHYSIOLOGICAL AGENTS ON THE ABILITY OF CELLS TO REPAIR AND RECOVER FROM RADIATION DAMAGE.

Clonogenic ability and growth curves were used to assess the influence of the selected agents on the cells. Because of space limitations, the results obtained may only be sumarised here. By dissolving Vitamin A (Retinol) in ethanol at an appropriate concentration, it was possible to introduce it into tissue culture media at levels up to 120 I.U./ml. Concentrations up to 30 I.U./ml did not greatly inhibit growth of CHO cells but higher levels did. Lower concentrations (≃ 1 I.U./ml) slightly enhanced the growth rate. Similar results were found in HeLa S-3 cells. Preliminary experiments suggest that these cells are sensitised to radiation by exposure to the vitamin. Hydrocortisone was easily dissolved in ethanol and introduced into the tissue culture media. Cell growth was reduced by high concentrations (≃ 100 µg/ml), undisturbed by medium concentrations (≃ 10 µg/ml) and enhanced at low levels (0.5 µg/ml).

The effect of PHA on HeLa cell growth was examined by Dr. E. Law, Pathology Department, University College, Dublin, in collaboration with the contractants. PHA was found to reduce the growth rate by 17% per 0.1 mg of PHA per ml of medium, in the concentration range up to about 0.5 mg/ml. This effect was reversible, even after 8 days culture in medium containing PHA. At higher concentrations evidence was found to suggest significant differences between batches of PHA.

A set of chambers and a gassing system have been constructed that will allow the concentration of oxygen in which cells are incubated be regulated. The system is versatile and allows up to 10 chambers be handled separately. Initial experiments suggest mild toxicity and reduced radiation repair after 24 hours incubation in gass mixtures containing less than 1000 p.p.m. oxygen.

The interpretation of the low dose region of survival curves is vital

in evaluating the contribution of repair and recovery to cell survival. This is particularly true when radiobiological models are used to assist rationalisation of the data. The development of many radiobiological models has proceeded without adequate quantitative or qualitative intercomparisons between them, and without a unified method to allow experimental points to be fitted to a particular model. Because of this we decided to investigate the possibility of producing a single statistical method that would allow the goodness of fit of each model to a particular set of data be evaluated. To this end the maximum liklihood method, solved by a simplex search technique, has been developed for use with a variety of models. The method ranks the models in order from the point of view of how well they fit the data. Anomolous results have been found, that may be a consequence of experimental design, rather than the fitting technique. It is hoped in the future to be able to quantify how well the various models fit a particular set of data, and to explore the possibility of generating a "confidence envelope" about a particular survival curve. This would indicate the bounds within which the curve should lie with particular confidence limits. The mathematical difficulties involved in the latter procedure are considerable and have not yet been fully explored.

Publications during 1975

Malone J.F., Hendry J.H., and Kinsella I.A.
Prediction of the Initial Shape of Survival Curves when direct measurement is not possible, in "Cell Survival after Small Doses of Radiation"
ed. T. Alper. p.313 - 319 (Chichester, Wiley).

Orr J.S., Laurie J., Kirk J., and Malone J.F.,
The "Pool" and the initial slope of survival curves for high and low LET radiation, in "Cell Survival after Small Doses of Radiation",
ed. T. Alper. p.86 - 88 (Chichester, Wiley).

Other 1975 publication relevant to Programme:
Malone J.F.,
"The Radiobiology of the Thyroid" in Current Topics in Radiation Research,
ed. M. Ebert and A. Howard p.263 - 368. (Amsterdam, Nth. Holland Pub. Co.).

Contractant de la Commission : Service de Radiobiologie du Laboratoire d'Enzymologie, C.N.R.S., 91190 Gif-sur-Yvette.
N° du contrat : 147-75-1-BIO-F
Chef du groupe de Recherche : Raymond DEVORET
Thème général du contrat : Radioresistance and repair in bacteria : the role of gene recA.

Importance of the recA gene.

Bacteria carrying a recA$^-$ mutation are very sensitive to ultraviolet (UV) and X-ray irradiations, they are unable to perform some important repair processes such as recombinational repair and induced reactivation (formerly called UV reactivation). Mutagenesis by UV light does not occur in recA$^-$ deficient bacteria and prophages are not induced either.

The many deficiencies conferred to bacterial cells by the recA$^-$ mutation indicates that the recA gene function plays a key role in the physiology of the cell. Therefore, the elucidation of the role of the recA$^+$ functions in repair and mutagenesis seems of prime importance.

The chromosomal region near recA codes also for other important cellular functions as exemplified by the mutations lexB and tif.

We aimed this year at determining the complementation pattern of all these mutations as well as their precise location.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques :

R. DEVORET, Ph. MORAND et A. GOZE

Titre du projet : Mutations in the recA region of E. coli K12 : Mapping and Complementation data.

Complementation tests

The complementation pattern of recA and lexB mutations was established by the construction of heterogenotes. Mutations recA and lexB were introduced by P1 transduction into the same F^- recipient genetic background and on derivatives of F143, an F-prime sex factor ; the various F-prime derivatives were then transferred to the newly constructed recipient strains.

Complementation was tested by the restoration of the resistance of the heterogenotes to UV light and X rays. The dominance or recessivity of the various recA and lexB mutations was estimated with the same test.

Table 1 : Dominance and complementation of various mutations in the recA region.

		Markers on the chromosome							
		W.T.	lexB30	rec-34	lexB31	recA13	recA1	recA36	recA11
Markers on the episome	W.T.	+	+	+-	+	+	+	+	+
	lexB30	+	-	-	+	+	+--	-	-
	lexB31	+	+	+	-	+	+--	-?	+
	recA13	+	+	+	+	-	-	-	-
	recA36	+	-	-	-	-	-	-	-

Marker location

The mapping of the mutations recA, lexB has been deduced from transduction experiments with either srl^+ (two point test) or srl^+ and $cysC^-$ (three point test). Both tests concurred in their results (see fig.1).

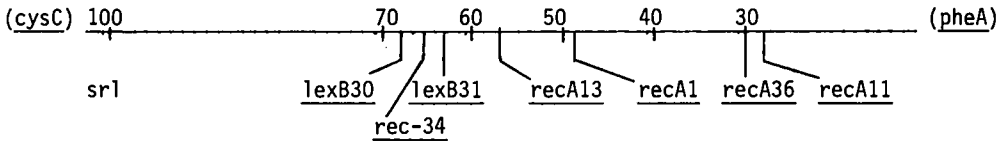


Figure 1 : Location of the recA alleles in percentage of cotransduction with srl at 37°.

the location of tif-1, based on cotransducibility of srl^+ with tif-1 at 30°, is indicated in fig.2. However, the location of some recA markers, like recA11, varies when the temperature of transduction changes. This peculiar effect was indicated to us by A.J. Clark for some other markers. Furthermore, the data of the three point test for tif-1 are at variance with those of the two point test. We are currently trying to resolve this difference.

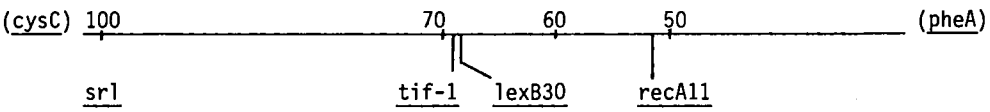


Figure 2 : Location of lexB30, recA11, tif-1 in percentage of cotransduction with srl at 30°.

Conclusions

The division of the recA region into two parts based on the difference in phenotypic expression of the mutations is supported by our mapping and complementation data : 1) recA13 complements all the lexB but not the recA markers studied ; 2) there are two clusters of mutations : recA and lexB ; 3) any mutation that increases the spontaneous production of phage from the resident prophage lies in the lexB sector (e.g. rec-34, lexB31, tif-1). The same situation is found with many alleles in the lexA region.

The validity of the mapping of recA mutations may be subject to caution because the recA function itself is required to map the recA mutations.

Despite the above-mentioned restriction, we are, nevertheless, encouraged to state the following. The pattern of complementation we obtained in the recA region leads to the suggestion that there is either intragenic complementation or complementation between polypeptidic chains coded by at least two cistrons.

We propose the hypothesis that the products coded for by the recA region form a complex which includes the lexA product(s) and that this complex is involved in DNA replication, recombination and repair in E. coli.

Contractant de la Commission : C.E.A- Centre d'Etudes Nucléaires.Fontenay-aux-Roses (France)

N° du Contrat :

Chef du groupe de recherches : Docteur R. LE GO

Thème général du contrat :

Study on R.B.E. helion beam preliminary to human radiotherapy use.

The study of the R.B.E. o a high energy helion beam was chosen as a preliminary approach of the possible application of heavy ions in radiotherapy, using the 645 MeV helion beam emitted by the Saturne synclotron.

The R.B.E. was studied simultaneously on several biological systems. Our laboratory had studied the chromosomal aberrations in normal human lymphocytes.A number of rotating copper plates of various thickness were placed in the monoenergetic helion beam in order to transform the Bragg peak and to have a constant dose distribution at a certain depth in tissue equivalent medium. The area where the dose distribution remained fairly constant was called "plateau". The dose distribution did not remain accurately constant but presented 9 little peaks.

Blood samples included in extrat-flat plexiglas containers were irradiated at different places in the beam :

For dosimetry purpose , two ionisation chambers were set in the beam : a monitoring chamber and a tissue equivalent extrapolation chamber.

For each blood sample, several culture tubes were used.The cultures were incubated at 37°C for 46 to 50 hours.

Dicentrics, rings and fragments were detected by direct microscope examination.

Résultats du projet n°

Chef du projet et collaborateurs scientifiques:

Docteur R. LE GO, Collaborateur scientifique: Mme DOLOY

Titre du projet :

Dose effect relationship for in vitro irradiation of human lymphocytes (chromosome aberrations).

For each position in the beam, the number of irradiated blood samples and the number observed cells are listed in Table I

Place of sample	Number of blood samples	Number of observed cells
entrance in medium	IO	2 800
beginning of plateau	4	I 234
middle of plateau	I3	3 475
end of plateau	II	2 875
total	38	IO 384

The studied dose range for each position in the beam was comprised between IOO and 500 rads.

The results showed a certain variability probably due to the instability of the physical characterictics of the beam throughout the experiments. This instability might involve LET variations and an unaccuracy in the dose to samples, chiefly during the first experiments for wich the dosimetry was not made at the same time as the irradiation of blood samples.

The Table II shows the dose-effect relationships calculated for the various positions.

Our experiments are being continued in order to specify the dose-effect relationships and to study the effect of an intermittent dose distribution.

The interest of such radiation for radiotherapy can not be made clear, so long as, the results supplied by the various biological systems investigated have not been compared.

Hélions 645 Mev

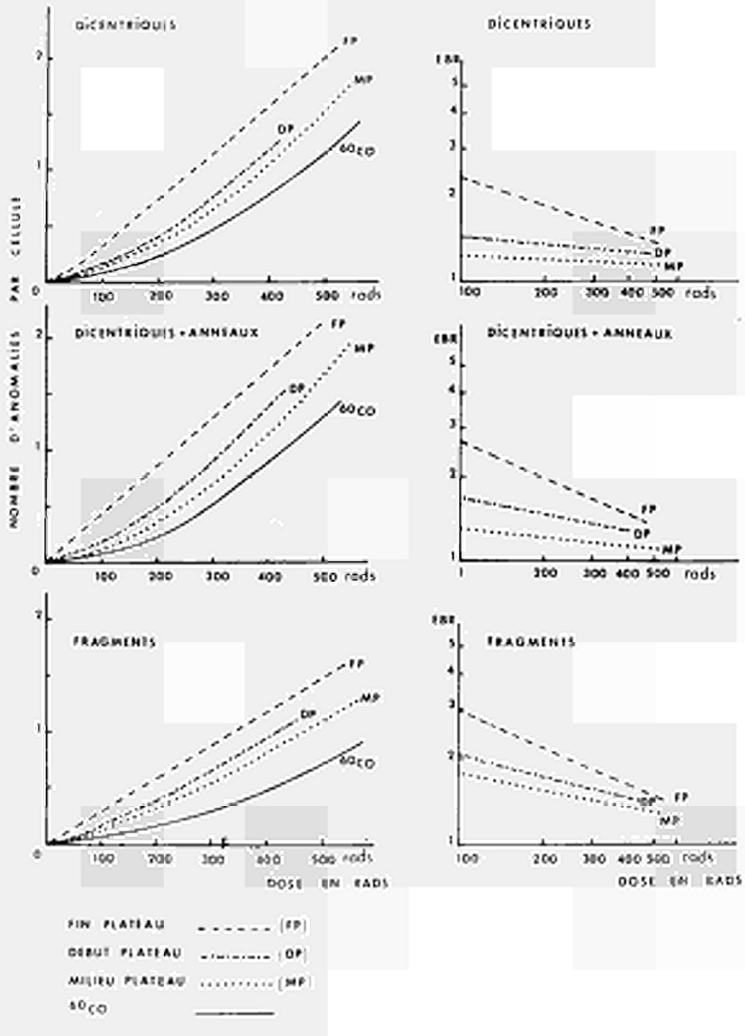


TABLE II

Contractor: Department of Radiation Genetics and Chemical Mutagenesis
(C.N.R.)

Contract No.: 136-74-7-BIOI

Head of Research Team: Prof. Dr. G. Olivieri

General subject of Contract: Studies on induced chromosome aberrations and chromosome structure

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In 1975 our group worked mainly along those lines of research involving: the mechanisms of formation, the transmissibility, the effects and the factors modifying the frequency and the type of chromosomal aberrations. These problems were tackled using the following test systems: human lymphocytes, aneuploid or endoreduplicated Chinese hamster cells in vitro, somatic and germ cells of Drosophila m..

The main results obtained concern:

- the study of factors affecting the pattern of rejoining (symmetric or asymmetric) in the formation of chromosomal exchanges. We noted that the relative frequencies of induced symmetrical and asymmetrical chromatid interchanges vary in relation to I) the cell cycle phase in which the aberrations are produced 2) the type of mutagen treatment used 3) the type of cell in which the aberrations are produced. The results obtained confirm our hypothesis (Olivieri et al. 1973) on the factors responsible for the type of rejoining and indicate the necessity of keeping these factors in mind when evaluating the genetic risk connected with a certain mutagen treatment. Parallel research gave results on the mechanisms of the processes of cell endoreduplication in vitro.

- the study of factors determining differences in radiosensitivity. We have investigated whether the variation with sex of irradiation-induced chromosome damage in somatic cells of D.m., remained both after treatment with methyl methanesulphonate (MMS) and in stocks with no crossing-over in the females.

The results obtained confirm our hypothesis (Gatti et al. 1974) that the differences in radiosensitivity between the two sexes is correlated with the mechanisms which in D. m. cause the presence of crossing-over only in the females.

- the influence of nutrition on radiosensitivity. We plan to study the influence of genetic variation or environmental changes on the frequency of induced aberrations. In this respect we are studying in D. m. the relationship between feeding and radiosensitivity. We have observed in cultures with a reduced amount of medium a direct correlation between body weight of the larvae treated, radiosensitivity and frequency of physiological crossing-over in the females.

- the relationships between chromosome structure and aberrations. Study was begun on the genome of D. m. and of other Dipterae using various cytochemical techniques. This year's research made possible the fine banding of the heterochromatin in the chromosomes in somatic cells and the interpretation of the distribution between chromosomes and within the chromosome of the aberrations induced by U. V. rays, X-rays or MMS.

- the relationships between chromosome aberrations and lethality. Using the test of chromosome aberrations in somatic cells, it is possible to correlate in D. m. the chromosomal and somatic damage, understood as lethality, induced at the larval stage. We have observed that where there are differences in the frequency of the aberrations induced (between males and females, between animals of different weights, in relation to particular treatments) there is a corresponding different frequency of induced lethality.

Project No. : 1

Head of Project and scientific staff: Drs. C. Tanzarella, Drs. R. De Salvia, Drs. A. Modesti, Mrs. E. Vitagliano

Title of Project: Variations through the cell cycle of the pattern of rejoining: experiments with human leucocytes

---

We plan to investigate the relative frequencies of X-ray induced symmetrical and asymmetrical chromatid interchanges as function of the cell cycle. In this project we intend to use various types of human cells. The first approach was made using leucocytes irradiated in vitro at various lengths of time after the initiation of the cultures.

Three experiments were performed in which different cultures were irradiated with 300 R (180 kV, 6 mA, 3 mmAL and 125 R/min) 53, 55, 57, 60, 65h after initiation of the cultures. The cultures were fixed at 72h. Parallel cultures were given pulses of ^{3}H T dR to confirm the stage of the cell cycle treated. In the three experiments there were no significant differences and therefore we were able to pool the data obtained. For the early S phase, out of 2262 metaphases were scored 102 symmetrical (Symm.), 124 Asymmetrical (Asymm.), 84 Triradials (T) with 45.1% Symm. out of the total Symm. + + Asymm. and 27.1% T of the total exchanges. For the medium S phase, out of a total of 3209 metaphases were scored 124 Symm., 211 Asymm. and 92 T with 36.8% Symm. out of the total Symm. + Asymm. and 21.4% of the total exchanges. For the late S and G_2 phases, out of a total of 1450 metaphases were scored 38 Symm., 24 Asymm., 17 T., with a percentage of Symm. of 61.2% of the total Symm. +Asymm. and of T. of 21.5% of the total exchanges. The ratio Symm. /Asymm. was therefore not constant in the various phases of the cell cycle, there being a clear prevalence of asymmetrical exchanges

in the medium S phase. Also of interest is the observation concerning the greater frequency of triradials at the beginning of the S phase.

Further experiments are under way to provide a better description of the variations of the rejoining pattern in the course of the cell cycle. Preliminary results indicate that further information can be obtained by inducing aberrations with chronic irradiation following the incorporation of ^{3}H T dR.

Project No.: 2

Head of Project and scientific staff: Dr. S. Pimpinelli, Dr. D. Pignone, Dr. M. Gatti, Prof. G. Olivieri

Title of Project: Chromatid interchanges and the cell cycle in Drosophila melanogaster

---

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. The main purpose of these studies was to compare the response of the homologous chromosomes of D. m. in somatic pairing with the response previously shown by the paired sister chromosomes in the diplochromosome in order to determine whether the rejoining pattern depends merely on the condition of pairing of the chromosomes or on a finer molecular organization of the 4 chromatids that comprise the two paired systems.

Three experiments were carried out, following the same scheme: nerve ganglia of third instar larvae of the Oregon R strain were irradiated with 1250 R x rays (180 kV, 6 mA, 3 mm Al). After 1.1/2, 3, 4.1/2 and 6h. the ganglia were fixed and squashed in acetic orcein. In addition a certain number of ganglia were treated for 15' with ^{3}HTdR(5 μCi/ml; Spec. Act 2 Ci/mM) and were then irradiated. After 1.1/2, 3, 4.1/2 and 6h. they were squashed and autoradiographed. All the slides obtained were coded before observation.

Since no significant differences between the three experiments were observed, the results have been pooled. Fig. 1 shows the relative frequency of the symmetrical exchanges in relation to the asymmetrical ones at the various times of post-irradiation fixation; Fig. 1 also shows the data relative to the autoradiographic analysis.

The results obtained, taken as a whole, indicate that in exchanges

between autosomes during the S phase, both in the females and in the males, the asymmetrical type of rejoining is preferred; this prevalence later becomes reduced as the cell approaches mitosis. On the other hand, in the exchanges between X-chromosomes in the females there are no significant variations in the type of rejoining through the cell cycle, there being a slight but constant preference for symmetrical rejoining.

The present data confirm the hypothesis that the type of rejoining is decided at molecular level rather than depending on the arrangement or physical proximity of the chromosomes.

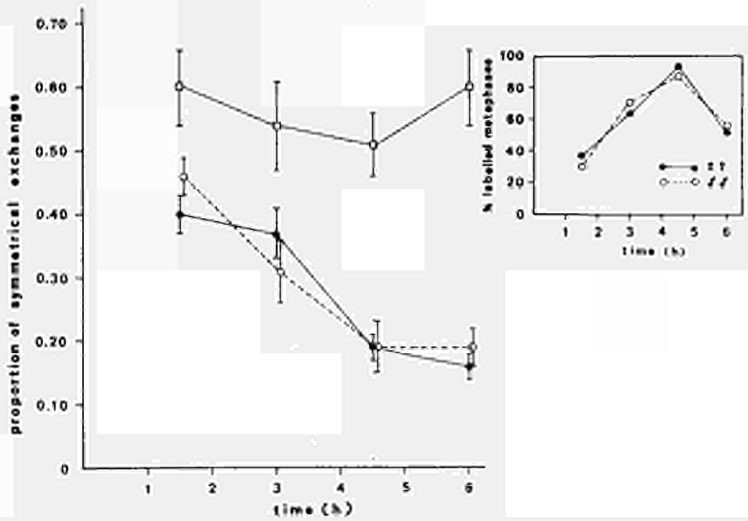

Fig. 1 - Relationship between labelling index and the proportion of symmetrical exchanges at the various sampling times after irradiation. (□——□ ♀♀ sex-chromosomes; ●——● ♀♀ automosomes; o- - -o ♂♂ autosomes).

Project No. : 3

Head of Project and scientific staff: Dr. R. Ricordy, Dr. F. Spirito

Title of Project: Variations through the cell cycle of the pattern of rejoining: experiments with Chinese hamster in vitro and in vivo

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We plan to investigate how the symmetrical/asymmetrical chromatid exchanges differ in the various phases of the cell cycle in vivo and in vitro. To this end two experiments were carried out in vitro by irradiating a clonal derivative of the CHEF 125 cell line of Chinese hamster. In these experiments 125R were given to cell cultures in logarithmic growth and subsequently various cultures were fixed with intervals of 2h between them up to 20h after irradiation. Parallel cultures were given pulses of ^{3}H TdR to confirm the cell cycle phase treated. There were no significant differences in the two experiments. Therefore we were able to pool the data obtained.

On the whole, for the various points examined, out of 2000 metaphases 485 interchromatid exchanges were scored. In the different phases of the cell cycle the ratio symmetrical/asymmetrical interchanges never diverged significantly from 1:1. There was also observed a greater frequency of triradials at the beginning of the S phase. The results obtained on this aneuploid cell line in vitro differ, as far as the ratio Symm/Asymm. is concerned, from the results obtained in analogous experiments in other test system (Vicia faba, D.m., human leucocytes). It will be interesting to note whether this difference will be confirmed in the irradiation in vivo of the animal as a whole. This type of analysis is being undertaken by Dr. A.T. Tates and P.V.Buul in Leiden with whom we are collaborating.

Project No.: 4

Head of Project and scientific staff: Dr. S. Pimpinelli, Dr. A. De Marco, Dr. M. Gatti, Prof. G. Olivieri

Title of Project: Variation with sex of chromosome damage in somatic cells of D. m.: experiments with Oregon R and C3G stock

---

So that we might study the possible correlation between the variation with sex of irradiation-induced chromosome damage in somatic cells of Drosophila m. and the presence or otherwise of crossing-over in the two sexes, experiments were performed using a stock homozygote for the gene C3G which, as is known, drastically reduces crossing-over in the females.

Larvae at the third stage, both of the Oregon R stock and of a stock homozygote for C3G, were irradiated with 625 R. Then the chromatid aberrations induced in the nerve ganglia were examined. In the three experiments performed with the same procedure, on the whole about 6000 metaphases were examined. The results were similar in the three experiments and confirmed a greater frequency of aberrations in the females than in the males of the Oregon R stock in the ratio of about 3:2. There was not, however, any significant difference between females and males in the C3G stock. Also the frequency of aberrations in the two sexes for the C3G stock is similar to the frequency found in the males of the Oregon R stock.

These results would indicate a) that the mechanisms which allows the crossing-over in the females play a role in the transformation of potential chromosomal lesions, increasing, when present, the frequency of the chromosomal aberrations; b) that a meiotic mutant can act also in somatic cells.

Project No.: 5

Head of Project and scientific staff: Dr. M. Gatti, Dr. S. Pimpinelli, Dr. A. De Marco, Drs. C. Tanzarella

Title of Project: Comparative study of chromosomal aberrations induced by methyl methanesulfonate(MMS) and X-rays in somatic cells of D. melanogaster

---

The pattern of chromosomal aberrations induced by MMS in gangliar cells of D. melanogaster has been studied with the aim of comparing it with the pattern of aberrations induced by X-rays. Oregon R strain larvae at the third stage were exposed to MMS vapours for 25 or 30 minutes. These larvae were then dissected and the nerve ganglia fixed with the usual procedure (Gatti et al., Genetics 1974) after 4, 8 and 12 hours. For each fixing time, 1000 male metaphases and1000 females were scored. The cells fixed 4h after treatment showed very few aberrations while about 30% of those fixed at the subsequent times showed chromosomal damage. Most probably this phenomenon results from the fact that MMS has an S-dependent effect. The following similarities and differences between MMS and X-rays emerged from an analysis of the aberrations induced:

1) Both mutagens do not induce intraexchanges.

2) For both mutagens there is a greater frequency of aberrations in the females than in the males; for MMS the difference in sensitivity between the two sexes is, however, more marked.

3) MMS displays greater specificity than X-rays in inducing breaks on the heterochromatic areas of the X chromosome and the autosomes, whereas it seems to be less efficient than X-rays in breaking the Y chromosome, which, as is known, is entirely heterochromatic.

4) The chromatid exchanges induced by MMS are mainly of the symmetrical type. In that the symmetrical exchanges are transmitted from one cell generation to the next, while the asymmetrical ones are one of the main causes of cell lethality, this latter fact would go to indicate that, for equal induced chromosome damage, the long-term genetic risk from alkylating substances is greater than that from X-rays.

Project No.: 6

Head of Project and scientific staff: Dr. M. Gatti, Dr. S. Pimpinelli, Dr. A. De Marco, Dr. R. Ricordy

Title of Project: Comparative study of chromosome aberrations induced by methyl methanesulfonate (MMS), nitrogen mustard and X-rays in endoreduplicated Chinese Hamster cells

---

X-rays given during the S phase cause prevalently asymmetrical exchanges between chromosomes of D. melanogaster with somatic pairing and symmetrical exchanges within the diplochromosomes. The alkylating agents, however, produce in D. melanogaster a prevalence of asymmetrical exchanges. This study was undertaken to ascertain what kind of exchanges the alkylating agents induce within the diplochromosomes. To this end cells in the G1 phase which precedes the second cycle of DNA replication in the process of endoreduplication were treated for 45 minutes with two doses of MMS and of nitrogen mustard. These cells were then fixed after 24 and 28 hours. On the whole 800 endoreduplicated methaphases were analyzed for a total of 620 exchanges. From this analysis it emerged that both mutagens induce a strong prevalence of exchanges of the symmetrical type (the ratio Symm/Asymm was $\sim$ 20:1). These data, together with the above, would indicate that:

a) After X-ray treatment during the S phase the rejoining is decided at molecular level and depends mainly on the respective polarity of the subunits which participate in the exchange (Olivieri et al., 1973).

b) After treatment with alkylating agents, the rejoining depends mainly on the arrangement of the chromosomes; these mutagens induce rejoining, preferably of the X type, in all paired structures (chromosomes paired somatically, diplochromosomes).

Project No.: 7

Head of Project and scientific staff: Dr. S. Pimpinelli, Dr. M. Gatti, Dr. D. Pignone, Prof. G. Olivieri

Title of Project: Chromosome aberrations induced by U.V. rays in somatic cells of Drosophila m.

We are planning to investigate the induction by U.V. rays of chromosomal aberrations in somatic cells of D.m. Our aim is that of comparing the types of aberration induced by U.V. rays and their distribution between the various chromosomes and within the chromosomes with the aberrations produced by other mutagen treatments (X-rays, MMS, etc.).

We therefore exposed to U.V. rays from a germicide lamp larval ganglia of D.m. which were fixed 6 and 9 hours after exposure. The preliminary results obtained refer to the scoring of 1004 male cell metaphases in which the aberrations found are distributed thus:

40 isochromatid deletions (30 in the autosomes and 10 in the X chromosome); 55 isochromatid deletions (19 in the autosomes, 4 in the X chromosome, 32 in the Y chromosome); 14 exchanges (11 symmetrical, 3 asymmetrical). It was also noted that about 85% of the aberrations involve the centromeric heterochromatic regions of the various chromosomes. These data, even if preliminary, indicate a marked difference in the pattern of aberration caused by U.V. rays with respect to that of X-rays. The marked sensitivity of the Y chromosome to the action of U.V. rays and the clear prevalence of the aberrations in the centromeric regions are of great interest.

Project No.: 8

Head of Project and scientific staff: Dr. A. De Marco, Drs. O. Venezia, Mrs. M. Belloni

Title of Project: Correlations between body weight, radiosensitivity and frequency of crossing-over in D. m.

We are planning to investigate the modifying effect of genetic and environmental factors on the activity of various mutagens. Within this line of research, the present project will have the aim of studying in D. m. the role of different nutritional availability in the response to X-rays.

To this end different numbers of larvae were let grow in vials all containing the same amount of food. In relation to the different degrees of crowding, larvae are obtained with a body-weight which varies in the males from 0.9 to 1.8 mg and in the females from 1.2 to 2.2 mg. In further experiments females and males larvae, of different weights were irradiated with 625 R (180 kV, 6mA, 3mm Al, 125 R/min.). Of the larvae examined 65 were females and 54 males and in each of these were scored the aberrations produced in no fewer than 70 ganglia cell metaphases. Both in the females and in the males, there was a positive correlation ($P < 0.01$) between body-weight and chromatid aberrations. In fact, the heavier larvae have a greater percentage of aberrations.

Because of the known hypotheses which have been put forth with regard to the possible similarity of the enzyme systems attributed to the crossing-over and to the repair and transformation of chromosomal damage, we have felt it to be of interest to investigate the frequency of the physiological crossing-over when body-weight of the larvae is varied.

Thus we examined the frequency of crossing-over in females of different weights, heterozygotes for the markers b cn vg bw. Out of a total progeny of 8435 individuals deriving from the heavier females a total frequency of crossovers of 52.1% was obtained for the various zones of exchange, against a frequency of 47.2% of the progeny of 7264 individuals deriving from lighter females. These data on the whole could indicate that animals with a lighter body weight have at their disposal a reduced level of the enzymes necessary for physiological crossing-over or the transformation of chromosomal damage.

Project No.: 9

Head of Project and scientific staff: Dr. A. De Marco, Drs. O. Venezia, Mrs. M. Belloni, Drs. C. Tanzarella

Title of Project: Studies on correlations between chromosome damage and lethality

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With the perfection of new techniques which make it possible to directly investigate the chromosomal aberrations produced in somatic and germinal tissues of Drosophila m., it is easier to study in this organism the possible correlations between chromosomal aberrations and cell damage understood as death of the entire organism. We have therefore begun a program which provides for the analysis of mortality before hatching in D. m. larvae subjected to various mutagen treatments. In that as far as chromosomal aberrations induced by X-rays and by MMS are concerned, parallel studies carried out in our laboratory showed differences between the two sexes and differences correlated with body weight, we are investigating whether or not these differences persist also for lethality. Experiments using both X-rays and MMS on larvae of both sexes and of different body weight have indicated the existence of a relationship between chromosome damage and lethality. This correlation, however, is not present in a number of experiments because of the probable action of other factors which have not yet been properly checked.

Project No.: 10

Head of Project and scientific staff: Dr. M. Gatti, Dr. S. Pimpinelli, Dr. G. Santini, Dr. A. De Marco

Title of Project: Characterization of heterochromatin of various species of the genus Drosophila

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With the aim of studying the different radiosensitivities of chromatid regions defined by different cytochemical characteristics, we have characterized the heterochromatin of D. melanogaster, D. simulans, D. virilis, D. texana, D. hydei, D. ezoana, with the following techniques:

1) differential staining with the fluorochromes Hoechst 33258 and quinacrine,

2) selective decondensation by way of treatment of live neuroblasts with Hoechst 33258,

3) differential staining with Giemse after acid hydrolysis at 96°C (N-band method).

Correlating the results obtained with the base composition of the satellite DNA contained in the heterochromatin of the various species, we were able to establish that the areas which were fluorochrome bright with H 33258 and those decondensed by H 33258, all contain an AT rich DNA. There is not, however, always a correspondence between fluorochrome bright areas and decondensed areas. There are even very AT rich heterochromatic areas which are neither fluorescent nor decondensed. The different response of DNAs similarly rich in AT is in all probability due to the fact that they are coated in the various species with different chromosomal proteins. To this end it was found that the positive areas in N-band preparations identify along the chromosome a crowding of acid proteins capable of dulling the fluorescence of the Hoechst 33288 and of the quinacrine.

Publications

1) PIMPINELLI S., GATTI M., AND DE MARCO A., 1975: Evidence for heterogeneity in heterochromatin of Drosophila melanogaster. Nature 256: 335-337.

2) GATTI M., PIMPINELLI S., DE MARCO A. AND TANZARELLA C., 1975: Chemical induction of chromosome aberrations in somatic cells of Drosophila melanogaster, Mutation Res. 33: 201-212.

3) DE MARCO A., COZZI R. AND TOTI L., 1975: Cytological observations on mitotic and meiotic pairing in males of Drosophila melanogaster with In (1) sc^4sc^8. Genetica 45 (in press).

4) PIMPINELLI S., PIGNONE D., GATTI M. AND OLIVIERI G., 1975: X-Ray induction of chromatid interchanges in somatic cells of Drosophila melanogaster: variations through the cell cycle of the pattern of rejoining. Mutation Res., (in press).

5) GATTI M., PECCI L. AND OLIVIERI G., 1975: Spontaneous endoreduplication in chinese hamster cell cultures I.: effect of growth conditions. Caryologia,(in press).

6) GATTI M. AND OLIVIERI G., 1975: Spontaneous endoreduplication in chinese hamster cell cultures II.: analysis of the mitotic cell cycle. Caryologia,(in press).

7) GATTI M., PIMPINELLI S., SANTINI G.F. AND DE MARCO A.: Metodi citologici per la identificazione e la localizzazione del DNA satellite leggero in varie specie del genere Drosophila. Atti AGI XXI, 1975.

8) PIMPINELLI S., SANTINI G.F. AND GATTI M., (Roma): Caratterizzazione dell'eterocromatina di Drosophila melanogaster. Atti AGI XXI, 1975.

9) MUTATION RESEARCH GROUP OF GENETICS, Rome (Italy): Somatic cells of Drosophila melanogaster used as mutagenicity test system "in vivo" for investigation of induced chromosome aberrations. Vth Annual Meeting of the European Environmental Mutagen Society, Firenze 1975 (Poster).

10) MUTATION RESEARCH GROUP OF GENETICS, Rome (Italy): The problem of rejoining (symmetric or asymmetric) in the formation of chromosome aberrations. Vth Annual Meeting of the European Environmental Mutagen Society, Firenze 1975 (Poster).

11) DE MARCO A., BELLONI M.P., PIMPINELLI S. AND COZZI R.: Influence of nutrition on genetic damage induced by ionising radiation in D.m., Atti AGI XXI, 1975.

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STUDY OF THE ENZYMES OF DNA REPAIR IN HUMAN CELLS

- A. Falaschi
- F. Nuzzo
- S. Spadari
- M.A. Pedrini
- U. Bertazzoni (from 1.9.1974)

The activity of the past two years may be summarized in the following points:

1) Rapid assay system of repair ability - We have developed a simple assay of U.V. repair ability that measures U.V.-induced DNA synthesis in human cells in the presence of hydroxyurea. The assay has been applied to unstimulated human lymphocytes and allows to recognize the defect in the classical and De Sanctis Cacchione types of Xeroderma Pigmentosum (X.P.).

2) Variations of DNA enzymes in human lymphocytes - We have observed pronounced increases of the levels of some enzymes of DNA metabolism during the stimulation of human lymphocytes with phytohemoagglutinin (PHA). A first wave of increase is observed in correspondence to the wave of DNA synthesis (between the third and fifth day); a second wave occurs at later times (6th to 8th day) when DNA synthesis is decreased to very low values. In coincidence with the second wave, an increase of repair ability is observed, by the technique reported under 1).

3) Levels of DNA enzymes in X.P. and other syndromes - The levels of DNA polymerase, ligase, a DNase acting on single stranded DNA, a "nickase", and DNA kinase have been measured in fibroblasts from nine X.P. cases belonging to three different complementation groups, from one case of Bloom's syndrome, from one case of meiotic alterations and from nine normal controls. The variations of the levels in the normal population are very pronounced. The values of the enzymes in the pathological fibroblasts lie within those of the controls.

4) Purification of DNA enzymes from heteroploid human cells - We have purified from cells of the EUE line the DNA polymerases, the DNA ligase and three different DNases. For DNA polymerase, we observe the α and β (large and small) enzymes observed by many other authors. For ligase, we have demonstrated two forms of the enzyme, with different pH optima, one being probably a dimer and the other a monomer of the same structure. We have also described a number of other properties of the same enzyme. We have purified three different DNases acting on single stranded DNA. One is an exonuclease and corresponds probably to DNase III. The other two are endonucleases and are clearly distinct from the other endonucleases described sofar in mammalian cells. One of the endonucleases has also a pronounced "nickase" activity.

Future work

1) We intend to improve the rapid U.V. repair assay in view of its possible applicability to detect individuals deficient in DNA repair for U.V. damage; we intend to set up a new simpler assay for the repair of X ray damage.
2) We intend to distinguish which of the different isozymes (of DNA polymerase and DNase) increases in each of the two waves of induction after PHA stimulation of the lymphocytes.
3) We intend to assay cells from other diseases of DNA metabolism (other complementation groups of X.P., progeria, Fanconi's anemia, etc) for the levels of DNA enzymes; we intend also here to distinguish, where possible, the different isozymes. We shall try to obtain established lines having the repair defects of X.P. or other syndromes.
4) We intend to pursue the characterization of the different purified DNases, to check their possible involvement in repair function. We intend to study also the DNases acting on double stranded DNA, with particular attention to those that may be specific for damaged DNA.

Main publications:

S. SPADARI, G. CIARROCCHI and A. FALASCHI - Purification and properties of a polynucleotide ligase from human cell cultures. Eur. J. Biochem., 22: 75-78 (1971).

A. M. PEDRINI, F. NUZZO, G. CIARROCCHI, L. DALPRA' and A. FALASCHI - Induction of polynucleotide ligase in human lymphocytes stimulated by phytohemoagglutinin. Biochem. Biophys. Res. Commun., 47: 1221-1227 (1972).

G. C. F. PEDRALI NOY, S. SPADARI, G. CIARROCCHI, A. M. PEDRINI and A. FALASCHI - Two forms of the DNA ligase of human cells. Eur. J. Biochem., 39: 343-351 (1973).

A. M. PEDRINI, L. DALPRA', G. CIARROCCHI, G. C. F. PEDRALI NOY, S. SPADARI, F. NUZZO and A. FALASCHI - Levels of some enzymes acting on DNA in xeroderma pigmentosum. Nucleic Acids Research, 1: 193-202 (1974).

G. C. F. PEDRALI NOY, L. DALPRA', A. M. PEDRINI, G. CIARROCCHI, E. GIULOTTO, F. NUZZO and A. FALASCHI - Evidence for two waves of induction of DNA enzymes in stimulated human lymphocytes. Nucleic Acids Research, 1:1183-1199 (1974).

KURZZEITWIRKUNGEN (AKUTES STRAHLENSYNDROM UND SEINE BEHANDLUNG)

SHORT-TERM EFFECTS (ACUTE IRRADIATION SYNDROME AND ITS TREATMENT)

EFFETS A COURT TERME (SYNDROME AIGU D'IRRADIATION ET DE SON TRAITEMENT)

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Jahresberichten beschrieben:

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

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

099-BIAB ULB Bruxelles (Brachet)

095-BIOB CEN Mol (Maisin)

Vertragspartner der Kommission :

Assoziationsvertrag

zwischen

der Europäischen Atomgemeinschaft

und

der Association Claude-Bernard
dem Istituto di Ricerche Farmacologiche "Mario Negri"
dem Land Baden Württemberg
der Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek voor Gezondheit (TNO) und
der l'Université Libre de Bruxelles

Nr. des Vertrages : 088 - 72 - 1 BIAC

Leiter der Forschungsgruppen :

- Claude Bernard Association, Institut de Cancérologie et Immunogénétique, Villejuif : Prof. Dr. G. Mathé
- Istituto di Ricerche Farmacologiche "Mario Negri", Milano : Prof. Dr. S. Garattini
- Universität Ulm, Abteilung für Klinische Physiologie, Ulm : Prof. Dr. T.M. Fliedner
- Radiobiological Institute TNO, Rijswijk : Prof. Dr. D. W. van Bekkum
- Institut Jules Bordet, Bruxelles : Prof. Dr. H. Tagnon

Allgemeines Thema des Vertrages :

Consequences of Radiation Exposure : Prevention and Treatment of Pathological Effects

Allgemeine Darstellung der durchgeführten Arbeiten :

In 1975, the work of the laboratories participating in this association contract No. 088-72-1 BIAC was a continuation of the previous activities. They have been described in the Euratom Reports 1971 (EUR 4830 d-f-i-n-e), 1972 (EUR 4864 d-e-f-i-n), 1973 (EUR 5138 d-e-f-i-n) and 1974 (EUR 5332 d-e-f-i-n).

The collaborative work of the Association of 5 European research institutions is meant to contribute to the improvement

of existing and to the development of new approaches to prevent and treat pathological effects of ionizing radiation. Although radiation accidents in Europe have been up to now rare, there is increasing concern about the possible harmful effects of ionizing radiation in the scientific, industrial and medical use of nuclear energy. This concern is directed not so much against single, large exposures of human beings to ionizing radiation, but against low level repeated exposure to ionizing radiation from external sources or from incorporated radionuclides.

The research work performed in the framework of this contract is concerned with three problem areas :

1. Evaluation of damage caused by ionizing radiation
2. Treatment of hematopoietic failure

   and
3. Cell system research relevant to the first 2 topics.

The first problem area is of particular interest since there are only limited possibilities at hand to recognize harmful effects of ionizing radiation to the human organism when the radiation exposure is low and protracted over long periods of time. It is necessary to recognize minimal alterations in the function of the organism as well as its organs and organ systems at a time when pathological changes may still be reversible. These may, however, become irreversible if no therapeutic measures are taken (for instance, the eventual development of aplastic anemia or leukemia,the development of pulmonary fibrosis, etc.).

The second problem area is concerned with early and late consequences of one of the high-risk organ system The hematopoietic tissue is of particular importance since it is capable of tolerating quite a large

amount of ionizing radiation without failing to produce an adequate number of blood cells if the exposure is protracted over long periods of time. However, if additional burdens are imposed - such as chemical agents - the system may fail quickly. It is, therefore, important to investigate new approaches to treat hematopoietic failure after radiation exposure by stem-cell transfusion. This research problem - involving new techniques of cell collection, separation and storage, new approaches for the prevention and treatment of infectious complications and new considerations of the immunological mechanisms involved in the rejection processes after allogeneic tissue transplants - has stimulated the interest not only of those who need to be ready for the treatment of radiation injured persons. Research work in this area has had a considerable impact on the recent advances in cancer treatment (immunotherapy) as well as in organ and tissue transplantation in general.

The third problem area - cell system research - is devoted to research work the results of which are an essential prerequisite for the advancement of possibilities to recognize and evaluate as well as to treat radiation injuries. Only if knowledge of the kinetics, regulation and function of the hematopoietic and immune systems can be advanced can one expect progress in the first two problem areas. In this context, it appears essential to investigate drugs that are capable of modifying the responses of the immune system (for instance, to understand hematopoietic stem-cell regulation). The study of leukemic alterations of the hematopoietic system has proved to be very valuable in affording an understanding of the physiology of it.

These three major topics have been studied through close coordination and cooperation of the 5 participating research institutes in Villejuif, Milano, Rijswijk, Brussels and Ulm. These are the coregroups of the European Organisation for Research on Treatment of Cancer. It is mainly in this framework that the scientific members meet regularly to discuss their research programmes and to evaluate their results. Close cooperation has been present, particularly in the evaluation and treatment of hematopoietic failure as seen after exposure to ionizing radiation.

The basic work was considered and advanced in several research groups, such as the "stem-cell club" or the various committees of the "European Late Effects Project Group" (EULEP). Thus, the combined efforts of those participating in the execution of this contract represent a major European thrust in trying to improve existing or develop new tools to evaluate the damage observed after ionizing irradiation of human beings and to modify therapeutically the bone marrow failure seen after a variety of exposure conditions. The problem area "evaluation of damage caused by ionizing radiation" was of particular concern to the research group in Ulm. The toxic and teratogenic effects of tritiated compounds led to studies on the pathogenesis of such effects, including those seen at the sub-cellular level. On the other hand, the presence of hematopoietic stem-cells in the peripheral blood and the recognition of hematopoiesis as a complex of feed-back regulated cell renewal systems led to new concepts with respect to the possibilities of recognizing damage, not necessarily only when it is irreversible, but when it has merely caused increased activity in the cellular renewal aspect. The fact that the function of the hematopoietic stem-cell is of crucial importance to the function of hemopoiesis has lead the group in Rijswijk, as well as the group in Ulm, to investigate new approaches to characterize stem-cells in morphological as well as functional terms and with respect to their radiobiological properties. The groups in Brussels, Ulm and Villejuif are studying various aspects of leukemic cell proliferation and the underlying dysfunction of stem-cells, since it was felt that essential features of the normal system would become more apparent when they were studied in the diseased paradigm.

The problem area "treatment of hematopoietic failure" was studied experimentally as well as clinically in Villejuif, Rijswijk and Ulm and in certain basic aspects in Milano.

The group in Villejuif continued its extensive clinical programme - backed up by considerable animal research - to improve the results of allogeneic bone-marrow-transplantation in man. The greatest obstacle still is the inevitable graft-versus-host disease. Thus, some work is directed to the improvement of selection of donors (a large amount, however, to the immunological mechanisms involved). This group investigates the prevention and treatment of graft-versus-host disease at the preclinical and clinical level by a variety of means (use of anti-thymocyte serum, thymic chalone, anti-recognition-site serum, enhancing serum).

The group in Rijswijk concentrated its efforts on the methods of typing and matching for the various categories of tissue antigens in non-human primates and in dogs in order to explore the principles of selecting suitable donor-recipient combinations for bone marrow transplantation. This work requires extensive facilities for immunological and serological research in large animals. Furthermore, the group continued its work on the selective elimination of immune competent cells from the bone marrow, before grafting, by physical or immunological means and on the collection of purified hemopoietic stem-cell suspensions from adult or fetal hemopoietic tissue. In Rijswijk, extensive work was performed on the supportive care of radiation - exposed persons by means of bacteriological decontamination and by transfusion of cryopreserved blood platelets.

The group in Ulm continued its work at the preclinical and clinical level. In dogs, investigation into the establishment of a "blood-stem-cell bank" for the treatment of hemopoietic failure after ionizing radiation was completed. It is possible to restore hemopoiesis in lethally exposed dogs in the autologous as well as allogeneic situation by means of cryopreserved hemopoietic stem-cells collected by leukocytapheresis of the peripheral blood. This model is now ready for clinical testing and will be performed first in the autologous situation. Successful attempts have been made to concentrate blood

stem-cells and to reduce the contamination with lymphocytes by means of the discontinuous albumin gradient, as described first by the Rijswijk group. Extensive work was performed in the field of the characterization of leukocyte antigens in dogs as a basis for the selection of suitable donor-recipient combinations. In this area, extensive collaboration between the groups in Rijswijk and in Ulm as well as in München is underway. The supportive care of patients and animals with hematopoietic failure was extensively studied. In patients and in mice, the gnotobiotic approach was used. The organism was decontaminated by means of antibiotics and maintained in a gnotobiotic state within a germfree environment. In mice, the overall radiation mortality was reduced, especially that due to graft-versus-host disease after incompatible bone marrow grafting. There was virtually no death from secondary disease. Furthermore, leukocytapheresis was used in normal persons to collect granulocytes for transfusion into patients with hematopoietic failure.

The group in Brussels also employed granulocyte transfusions to overcome the dangerous phase of granulocytopenia in patients with hematopoietic failure.

The group in Milano contributes extensively in the field of stimulation of the immune system and by the characterization of new biological activities of radiomimetic and anti-tumoral compounds. The search for compounds that stimulate the various functions of the immune system may prove to be of particular relevance in the treatment of persons that have suffered only lightly from radiation exposure which, on the other hand, may have had a profound influence on the immune capacity. If this is impaired, neoplastic and non-neoplastic late effects may become apparent. It is hoped that the stimulation of the immune system would have a preventive action.

Last but not least, all groups performed basic research in order to shed more light on the kinetics, regulation and function of the hematopoietic as well as immunological cell renewal systems.

The group in Villejuif concerned itself with basic aspects of the immune system and with those mechanisms that inhibit and activate its functions. It thus complements the work of the group in Milano that studied new drugs that would stimulate immunological reactivity. This group tested the action of 2 new synthetic drugs and one of bacterial origin with respect to immunostimulatory capacity. Also of importance appears to be the work related to drugs capable of modifying the immunogenicity of tumor cells. Since neoplastic transformation is one of the possible late effects of ionizing radiation, the modification of the immunological reactivity of tumors may be of particular interest in radiation late effect studies. The group in Rijswijk is very much involved in the study of basic properties of hematopoietic stem-cells in man, monkeys, dogs and rodents. The major impact, however, was on histocompatibility typing for monkeys and dogs using novel approaches for typing and matching in the various categories of tissue antigens. The group in Brussels contributed to the study of the kinetics and regulation of proliferation of normal and pathological bone marrow cells. This group concerned itself with the question of the factors that determine the regulation of hemopoietic cell proliferation at various levels of cellular differentiation. The group in Ulm studied various basic aspects of hematopoietic cell renewal systems. Part of this work was executed in close collaboration with the group of Prof. Lucarelli in Pesaro. In the center of interest was the study of the characterization of the fetal, neonatal and adult hematopoietic stem-cell systems. The major question is that of competition of stem-cells in various states of activity and differentiation, such as cells "committed" to erythropoiesis, myelocytopoiesis or megakaryocytopoiesis. Here, radiomimetic drugs and radionuclides were used to evaluate the stem-cell activity during regeneration. In this context, interaction of normal and leukemic stem-cell proliferation and differentiation was used as a model for altered stem-cell function.

The research work carried out under the auspices of the present Association Contract represents a significant European contribution to the investigation of "short-term effects" of ionizing radiation and a basis for the consideration of repeated low level exposure, its evaluation and its treatment. Therefore, it is hoped that the combined effort of the participating institutions will be continued in 1976.

Contractant de la Commission: Association Claude-Bernard, Institut de Cancérologie et d'Immunogénétique.

N° du contrat: 088.72.I.BIAC

Chef du Groupe de Recherche: Professeur Georges MATHE, Directeur de l'Institut de Cancérologie et d'Immunogénétique.

Thème général du contrat: Prévention et traitement des états pathologiques secondaires à l'irradiation.

I - We have continued our work on experimental and clinical bone marrow transplantation.

Experimentally we have tried to prevent or cure the secondary disease which complicates the incompatible bone marrow grafts, and clinically followed our patients conditioned with anti-lymphocyte globulin.

II - We have starded the study of the hemopoïetic and immunologic effects of local irradiation.

III - We have set up a battery of tests for immune investigation of humans, which can be applied to the study of subjects irradiated accidentally as well as that of human hemopoïetic chimeras, the main risk of death being infection due to immune insufficiency.

Résultats du projet 088.72.I.BIAC
Chef du projet: Professeur Georges MATHE
Collaborateurs scientifiques: Léon SCHWARZENBERG -
Marcel HAYAT - Pierre POUILLART -
Titre du projet: Prévention et traitement des états patholo
secondaires à l'irradiation.

I - Bone Marrow Transplantation

1) Experimental

We have studied the effectiveness of six means for preventi curing the secondary disease due to graft-versus-host-reaction, three specific and three specific.

a) Effects of three non-specific agents: ATS, ATS-FAB fragm and thymic chalone.

Three nonspecific agents, anti-thymocyte serum (ATS) (table Fab fragment of ATS (table 2), and thymic chalone (table 3), known to bit acute GVH reaction, were compared for their efficacy in preventing curing secondary disease induced by the transplantation of parental bo marrow and blood cells into LD I00 irradiated F_1 mice (preclinical mod for studying clinical GVH). The agents were applied with the modalitie ble in man: incubation with the cells to be grafted, and early or late administration to the recipients.

ATS and Fab fragment increased neither the median survival nor the percentage of long-term survivors, whatever the modality of ad nistration.

The thymic chalone incubated with the cells prior to grafti increased singificantly the median survival time but not the percentag of long-term survivors. The early administration of thymic chalone to recipient increased significantly both the median survival time and th percentage of long-term survivors.

b) Effects of four specific agents: anti-recognition struct serum, host and donor-directed sera and host soluble H-2 antigens.

Four specific agents, anti-recognition structure serum (tab host (parental strain) and donor-directed sera (table 5 and 6), and ho

TABLE I

Effect of anti-thymocyte serum (ATS) on secondary disease in lethally irradiated (C57Bl/6xDBA/2) F1 mice receiving parental bone marrow and whole blood cells

T R E A T M E N T		:R E S U L T S					
		Median survival time in days (range)			% survival at I00 days		
		control group	treated group	statis-tics(2)	control group (1)	treated group	statis-tics(3)
In vitro	: INCUBATION OF THE GRAF-TED CELLS WITH ATS	60 (33-84)	40 (25-85)	N.S.	0	0	N.S.
In vivo	: EARLY TREATMENT : from day 0 to day +I4	27 (22-59)	34 (27-6I)	N.S.	0	I0	N.S.
Administra-tion of ATS	: LATE TREATMENT : from day +20 to day +34	53 (28-66)	46 (39-53)	N.S.	0	0	N.S.

(1) Controls were treated with normal rabbit serum

(2) Wilcoxon non parametric test; N.S. = non-significant ($P > 0,05$)

(3) χ^2 test; N.S. = non-significant ($P > 0.05$)

TABLE 2

Effect of Fab fragment from ATS on secondary disease in lethally irradiated (C57Bl/6xDBA/2) F1 mice receiving parental bone marrow and whole blood cells

TREATMENT		RESULTS					
		Median survival time in days (range)			% survival at I00 days		
		control group (1)	treated group	statistics (2)	control group (2)	treated group	statistics (3)
In vitro	INCUBATION OF THE GRAFTED CELLS WITH FAB	39 (2I-58)	46 (I6-64)	N.S.	0	I0	N.S.
In vivo Administration of Fab	EARLY TREATMENT AT DAY 0, +1, +2	36 (22-51)	36 (I6-64)	N.S.	0	10	N.S.
	LATE TREATMENT AT DAY +I4, +I5, +I6	32 (22-77)	33 (32-65)	N.S.	0	0	N.S.

(1) Controls were treated with I99 culture medium alone

(2) Wilcoxon non parametric test; N.S. = non-significant ($P > 0.05$)

(3) χ^2 test; N.S. = non-significant ($P > 0.05$)

TABLE 3

Effect of the thymic chalone on secondary disease in lethally irradiated (C57Bl/6xDBA/2) F1 mice receiving parental bone marrow and whole blood cells

TREATMENT		RESULTS					
		Median survival time in days (range)			% survival at I00 days		
		control group (1)	treated group	statistics (2)	control group (2)	treated group	statistics (3)
In vitro	INCUBATION OF THE GRAFTED CELLS WITH THYMIC CHALONE	25 (I9-4I)	40 (I3-80)	$P < 0.03$	0	I0	N.S.
In vivo chalone administration	EARLY TREATMENT from day 0 to +I0	33 (I2-57)	I50 (I2-390)	$P < 0.0I$	0	60	$P < 0.02$
	LATE TREATMENT from day +20 to +30	22 (8-35)	22 (20-60)	N.S.	0	0	N.S.

(I) Controls were treated with a kidney extract

(2) Wilcoxon non parametric test; N.S. = non-significant ($P > 0.05$)

(3) χ^2 test; N.S. = non-significant ($P > 0.05$)

TABLE 4

EFFECT OF ANTI-RECOGNITION STRUCTURE SERUM ON SECONDARY DISEASE IN LETHALLY IRRADIATED F1 (C57Bl/6xDBA/2) MICE RECEIVING C57Bl/6 PARENTAL BONE MARROW AND WHOLE BLOOD CELLS

TREATMENT		RESULTS					
		Medain survival in days (range)			% of survival at day I00		
		Controls	Treated	Statis-	Controls	Treated	Statistic(3)
IN VITRO	INCUBATION OF THE GRAFTED CELLS WITH ANTI RECOGNITION SITE SERUM	33 (2I-69)	35 (26-62)	N.S.	0	0	N.S.
IN VIVO ADMINISTRATION OF ANTI-RECOGNITION STRUCTURE SERUM	EARLY Daily, from day 0 to day +4	37 (I9-60)	37 (I6-64)	N.S.	0	0	N.S.
	LATE daily, from day +I4 to day +I8	37 (2I-75)	43 (30-68)	N.S.	0	0	N.S

(1) Controls were treated with normal F1 (C57Bl/6xDBA/2) serum
(2) Wilcoxon non-parametric test : N.S. = non-significant ($P > 0.05$)
(3) χ^2 test

TABLE 5

EFFECT OF HOST-DIRECTED SERUM ON SECONDARY DISEASE IN LETHALLY IRRADIATED F1 (C57Bl/6xDBA/2) MICE RECEIVING C57Bl/6 PARENTAL BONE MARROW AND WHOLE BLOOD CELLS

TREATMENT		RESULTS					
		Median survival in days (range)			% of survival at day 100		
		Controls	Treated	Statistic (2)	Controls (1)	Treated	Statistic(3)
IN VITRO	INCUBATION OF THE GRAFTED CELLS WITH HOST-DIRECTED SERUM	28 (15-36)	38 (19->360)	$P < 0.03$	0	30	N.S.
IN VIVO ADMINISTRATION OF HOST-DIRECTED SERUM	EARLY day 0	22 (20-34)	36 (27-48)	$P < 0.01$	0	0	N.S.
	LATE day +14	27 (20-30)	29 (22-35)	N.S.	0	0	N.S.

(1) Controls were treated with normal C57Bl/6 serum
(2) Wilcoxon non-parametric test; N.S. = non-significant ($P > 0.05$)
(3) χ^2 test

TABLE 6

EFFECT OF DONOR-DIRECTED SERUM ON SECONDARY DISEASE IN LETHALLY IRRADIATED F1 (C57Bl/6xDBA/2) MICE RECEIVING C57Bl/6 PARENTAL BONE MARROW AND WHOLE BLOOD CELLS.

TREATMENT		RESULTS					
		Median survival in days (range)			% of survival at day I00		
		Controls (1)	Treated	Statistic (2)	Controls (1)	Treated	Statistic (3)
IN VITRO	INCUBATION OF THE GRAFTED CELLS WITH ANTI-DONOR SERUM	39 (26-5I)	28 (6-57)	N.S.	0	0	N.S.
IN VIVO ADMINISTRATION OF DONOR-DIRECTED SERUM	EARLY day 0	23 (I3-32)	2I (9-35)	N.S.	0	0	N.S.
	LATE day +I4	27 (20-43)	27 (7-43)	N.S.	0	0	N.S.

(1) Controls were treated with normal DBA/2 serum

(2) Wilcoxon non-parametric test; N.S. = non-significant (P 0.05)

(3) ^{2} test

TABLE 7

EFFECT OF BALB/C SOLUBLE H-2 ANTIGENS ON SECONDARY DISEASE IN LETHALLY IRRADIATED F1 (C57Bl/6xDBA/2) MICE RECEIVING C57Bl/6 PARENTAL BONE MARROW AND WHOLE BLOOD CELLS

TREATMENT		RESULTS					
		Median survival in days (range)			% of survival at day I00		
		Controls (1)	Treated	Statistic (2)	Controls (1)	Treated	Statistic (3)
IN VITRO	INCUBATION OF THE GRAFTED CELLS WITH Balb/c SOLUBLE H-2 ANTIGENS	28 (I4-60)	I02 (23->478)	$P < 0.0I$	0	50	$P < 0.01$
IN VIVO ADMINISTRATION OF Balb/c SOLUBLE H-2 ANTIGENS	EARLY 3 injections/week, from day 0 to +21	24 (I4-47)	32 (I5-46)	N.S.	0	0	N.S.
	LATE 3 injections/week, from day +I4 to +35	24(4) (I4-47)	62 (I5->478)	$P < 0.05$	0	20	N.S.

(1) Controls were treated with C57Bl/6 soluble H-2 antigens
(2) Wilcoxon non-parametric test; N.S. = non-significant ($P > 0.05$)
(3) χ^2 test
(4) At day +I4 difference in mortality between treated and control groups is not significant (0% in treated group versus I0% in controls

TABLE 8

EFFECT OF C57Bl/6 SOLUBLE H-2 ANTIGENS ON SECONDARY DISEASE IN LETHALLY IRRADIATED F1 (C57Bl/6xDBA/2) MICE RECEIVING C57Bl/6 PARENTAL BONE MARROW AND WHOLE BLOOD CELLS

TREATMENT		RESULTS					
		Median survival in days (range)			% of survival at day I00		
		Controls (1)	Treated	Statistic (2)	Controls (I)	Treated	Statistic(3)
IN VITRO	INCUBATION OF THE GRAFTED CELLS WITH C57BL/6 SOLUBLE H-2 ANTIGENS	29 (22-37)	30 (27-34)	N.S.	0	0	N.S.
IN VIVO ADMINISTRATION OF C57Bl/6 SOLUBIE H-2 ANTIGENS	EARLY 3 injections/week, from day 0 to +2I	34 (23-48)	29 (12-53)	N.S.	0	0	N.S.
	LATE 3 injections/week, from day +I4 to +35	39 (2I-49)	39 (I9-5I)	N.S.	0	0	N.S.

(1) Controls were treated with I99 culture medium
(2) Wilcoxon non-parametric test; N.S. = non-significant ($P > 0.05$)
(3) χ^2 test

(parental strain) soluble H-2 antigens (table 7 and 8), were compared for their efficacy in preventing or curing secondary disease induced by transplantation of parental bone marrow cells and whole blood into LD I00 irradiated F1 hybrid mice. The agents were studied using methods applicable to man; i.e., they were either incubated in vitro with the cells to be grafted or administered into the hosts early or late after grafting. Anti-recognition structure serum and donor-directed serum did not increase median survival time or the percentage of long term survivors. Host-directed serum incubated with the cells before grafting or administered to the hosts early after grafting increased median survival but not the percentage of long-term survivors. Host soluble H-2 antigens prolonged median survival when administered to the hosts late after grafting, and increased both the median survival and percentage of long-term survivors when preincubated in vitro with the cells to be grafted.

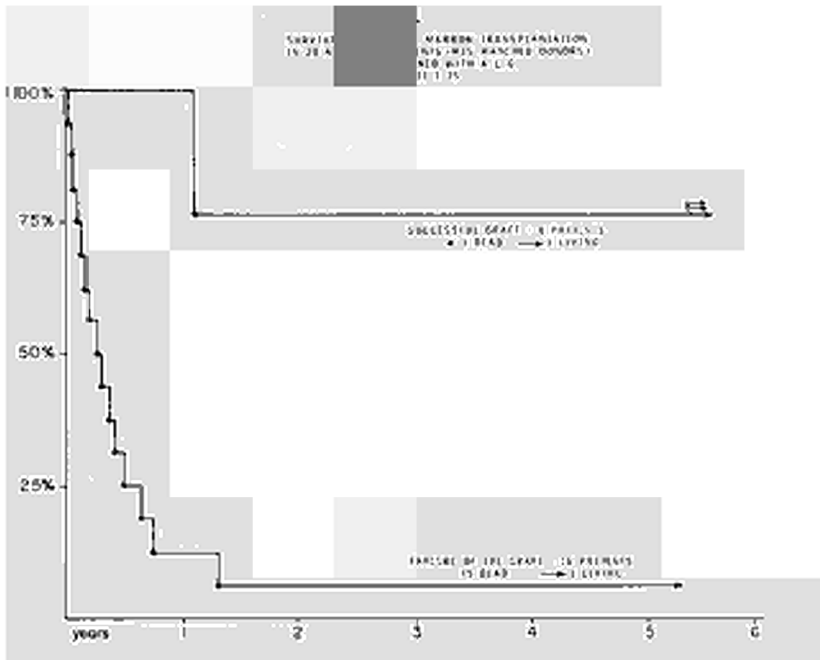
2) Clinical

Treatment of bone marrow aplasia by mismatched bone marrow transplantation after conditioning with antilymphocyte globulin; long term results.

20 patients with very severe bone marrow aplasia were submitted to mismatched bone marrow transplantation. Engraftment and transitory chimerism (from 2 to I0 months) not complicated by secondary disease were obtained in four patients: three are still alive after 5 years with a compensated haematological status. Engraftment was not obtained in I6 patients: only one is still alive after 5 years (fig.1).

II -Haematopoietic and immunologic effects of local irradiations

The lymphopenia and decreased PHA responsiveness seen in breast cancer patients receiving local irradiation led us to ask what other systemic effects might be expected from such treatment. Elucidation of the effects of local irradiation on immune responsiveness at distant sites should not only give insight into pertinent control mechanisms, but also provide a useful model for studying ways to restore the immune response in immunodeficient individuals. Accordingly, the left hind legs of $B6D2F_1$ mice were exposed to 4500 rads of x-irradiation in I0 sessions of 450 rads each over three weeks time. On the day following the last irradiation, or at suitable intervals thereafter, anti-SRBC, anti-TNP: KLH (TNP coupled to hemocyanin), and anti-TNP:POL (TNP.


100%
75%
50%
25%
years
1
2
3
4
5
6

coupled to polymerized flagellin) responses were tested, as well as the delayed hypersensitivity to picryl chloride, and the responsiveness of spleen cells to PHA (phyto-haemagglutinin) and PWM (pokeweed mitogen).

The responsiveness of these locally irradiated animals to SRBC can be characterized as follows: the number of direct PFC per spleen was significantly decreased one day (91% depression), 20 days (70%), and 29 days (43%) after the last irradiation; at 33 and 44 days, there were no differences between responses of experimental and control (untreated) spleens.

Similarly, anti-TNP: KLH (measured by indirect PFC) and anti-TNP:POL (direct PFC) responses were significantly diminished, by 51% and 67%, respectively, one day after the last irradiation. Four weeks later, no differences between spleens from treated and control animals for these two antigens were significant.

Delayed hypersensitivity to picryl chloride was measured by ear swelling seven days after sensitization. Animals sensitized to picryl chloride one day after the last irradiation had significantly less ear swelling upon challenge by the antigen than did unirradiated controls; when the animals were sensitized one month after irradiation, no measurab difference was observed.

Finally, while PHA responsiveness of spleen cells from locally irradiated mice was greatly depressed at all times tested (up to 50 days) PWM responsiveness was not depressed except on day 50 after the last x-ra treatment.

In conclusion, in these experiments a significant depression in immune response was found for both T-dependent and T-independent functions, and for both humoral and cell-mediated responses. Only the direct PFC response to TNP:KLH and the PWM responsiveness were found unchanged after the last irradiation. For most of the tests used, the results indicate that the immunodepression is transient and relatively short-lived, except for PHA responsiveness, which remained depressed seven weeks after irradiation, immune responsiveness was normal in all animals tested one month after termination of x-ray treatments.

III - Immune investigation in humans.

The tests which are now operational in our Institute are mention on table IX. Fig. 2 shows the immune insufficiency demonstrated in a hu bone marrow chimera while he was clinically well.

Fig. 2

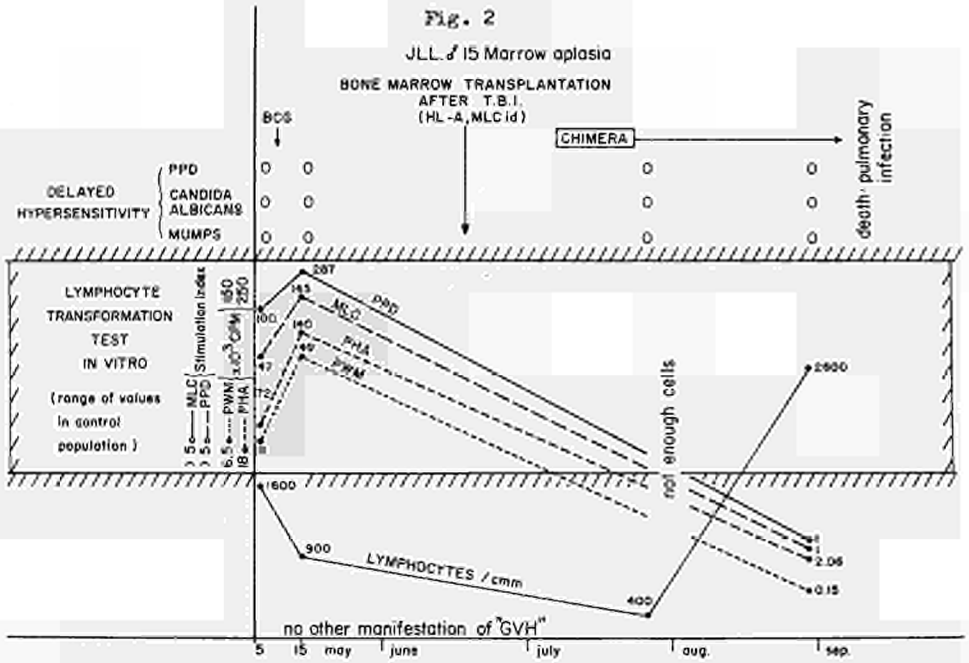


TABLE IX

Patient:
N°
Date :

IMMUNOLOGICAL INVESTIGATION

GENERAL RESPONSES	RESULTS
1. In vivo tests: skin reactions	
to recall antigens	Induration mm
Protein purified derivative (PPD)	
Candida	
Mumps	
Streptokinase-streptodornase	
to primary antigens	
Dinitrochlorobenzene (DNCB)	
Pichryl chloride	
Keyhole limpet haemocyanin (KLH)	
2. In vitro tests	
2.1 Routine counts in peripheral blood of	/mm^3
Lymphocytes	
Monocytes	
Immunoblasts	
2.2 Immunological and cytochemical counts with cell surface markers of	
T lymphocytes (spontaneous rosettes with sheep red blood cells)	/mm^3
B lymphocytes (membrane immunoglobulins)	
Monocytes (peroxydase positive)	
Null cells	

2.3 Functions of mononuclear cells:

2.31 Cell mediated immunity

Lymphocyte transformation by	CPM
Mitogens	
Phytohaemagglutinin (PHA)	
Pokeweed mitogen (PWM)	stimulatic index
Antigens	
KLH	CPM
PPD	CPM
Histocompatibility antigens (mixed lymphocyte reaction)	CPM
Lymphocytotoxicity	%
Against a permanent cell-line (51_{Cr} release)	
Due to K cells (L1210 leukaemia target cells)	
Suppressor cells(inhibition of PHA transformation)	CPM
Leukocyte inhibiting factor (LIF) in the serum	%

2.32 Humoral mediated immunity

Immunoglobulins serum concentrations	g/l
IgG	
IgA	
IgM	
Antibody titer	
Anti KHH	

PUBLICATIONS

FRIES D., GARNIER J., MARTIN B., LEON S. et SIMMLER M.C. Specific immunosuppression and histocompatibility in canine renal allografts. Transplant. Proc., I975, 7, 845

MARTIN M., HUCHET R., FLORENTIN I., HALLE-PANNENKO O., PROCYK S., PRITCHARD L. et MATHE G. Effect of local irradiation on cellular and humoral immunity. in "Fourth annual Conference, International Society for Experimental Hematology", Yougoslavie, Sept. 2I-24, I975 (abstract N°I32, p. 59).

MATHE G., HALLE-PANNENKO O. et BOURUT C. Potentialisation par administration de BCG d'une immunodépression induite par la cyclophosphamide. Applications aux transplantations. C.R. Acad. Sc., Paris, I975, 280, I749.

MATHE G., HALLE-PANNENKO O., FLORENTIN I. et KIGER N. Prevention and treatment of graft-versus host disease. Third Meeting of European and African Division, Londres, Août 24-28, I975 (abstract 11-20) International Society of Haematology.

PRITCHARD L., MARTIN M., HALLE-PANNENKO O. et MATHE G. Systemic effects of local irradiation on hemopoiesis in B5D2FI mice. in "Fourth annual Conference, International Society for Experimental Hematology", Yougoslavie, Sept. 2I-24, I975 (abstract N°76, p.34)

SOUS PRESSE

HALLE-PANNENKO O., ABUAF N. et MATHE G. Use of a soluble histocompatibility antigen in the control of transplantation reactions. Transplant. Proc., I975, sous presse.

HALLE-PANNENKO O., ZALC-GOUJET C., KUROIWA A., BOURUT C. et MATHE G. Prevention and treatment of secondary disease. II. Effects of four specific agents: antirecognition structure serum, host and donor-directed sera and host soluble H-2 antigens. Int. J. Radiol. Oncology, Biology, Physics, I975, sous presse.

PUBLICATIONS ~~(suite 1)~~

MATHE G., HALLE-PANNENKO O., KIGER N., FLORENTIN I. et BOURUT C. Prevention and treatment of secondary disease. I. Effect of three non specific agents: ATS, ATS-Fab fragment and thymic chalone. Int. J. Radiol. Oncology, Biology, Physics, I975, sous presse.

SIMMLER M.C. et BRULEY M. Immunodeficiency and immunorestoration. Cancer Immunol. Immunoth., I976.

SIMMLER M.C., SCHWARZENBERG L. et MATHE G. Attempts at non specific cell-mediated immunorestoration of cancer patients with BCG. Cancer Immunol. Immunoth., I976.

Istituto di Ricerche Farmacologiche "Mario Negri"

Contract Number : 088-72-1 BIAC

Head : Silvio Garattini, M.D.

CONSEQUENCES OF RADIATION EXPOSURE, PREVENTION AND TREATMNET OF PATHOLOGICAL EFFECTS.

A first line of investigation followed during this contract period has concentrated on the search and characterization of the mechanism of action of new immunostimulatory agents. It is known that one of the most radiation-sensitive systems in the body is the immune one and agents of this kind have the potential of decreasing this type of radiation exposure consequences; in addition, much current interest is attached to the use of immunoadjuvants in combination with classical treatments in the therapy of tumors. Three new agents, 2 synthetics and one of bacterial origin, have been found to possess definite immunostimulatory capacity in animals employing a series of tumorous and non tumorous systems. Indications of differences in the immunocyte subpopulation(s) involved have also been obtained, which hopefully will permit a more rationalized and effective integration of these agents in new therapeutic strategies. Connected with this line, are studies stemming from previous work supported by this contract on the synergism of selected, complementary immunotherapeutic manoeuvers based on the knowledge of the mechanism of action of immunoadjuvants. The combination of C.parvum

and specific anti-tumor arming serum is presented as a first example of such heretofore not investigated therapeutic approach.

A second major line of studies involved the further characterization of a novel biological activity possessed by radiomimetic and antitumoral compounds, i.e. the capacity to modify tumor cell immunogenicity. As preliminary phases for a possible therapeutic exploitation of this phenomenon, the minimal treatment conditions for inducing increased neoplasm immunogenicity have been defined for a series of experimental models and the possibility of reconstituting the depressed immune capacity of the modified tumor bearing-host were successfully explored. In addition to shedding further light on the possible biological mechanisms at the basis of this activity of radiomimetics it is believed that this type of findings may open the road to a novel type of antitumoral immunotherapeutic intervention.

Results of project No. 1

Investigators : F.Spreafico, M.D., A.Tagliabue, Ph.D., A.Vecchi, Ph.D.

Title of project : SEARCH FOR NEW IMMUNOSTIMULATORY COMPOUNDS

Immunostimulatory capacity has been recognized for two new substances and initial characterization of this biological activity has been obtained. The first is a synthetic agent /3-(p-chlorophenyl)-2,3-dihydrothiazolo (3,2-a)-benzimidazolo-2 acetic acid/ and has been observed to induce an increase in the primary antibody response to standard antigenic stimuli (allogeneic erythrocytes, soluble proteins) of normal nice after optimal and suboptimal challenges. Increase in the peak responses as well as prolongation of the response after single injection of 20-50 mg/kg were found. The dose-response curve appears to be steep and indication of paradoxical responses at supraoptimal doses were seen. This compound is capable of stimulating also cell-mediated reactivity and a protective activity was seen also in leukemia-lymphoma and solid murine tumor models. In fact when administered in conjunction with irradiated tumor cells, cures in a significant porportion of tumor-bearing animals were seen in the L1210 and L5MF-22 lymphomas. Significant antimetastatic activity was found in the Lewis lung carcinoma model even when treatment was started at an advanced tumor stage, and a remarkable synergism with previous chemotherapy could be evidenced. In addition, this compound was capable of significantly shortening the immune negativity period induced by chemical immunosuppressants.

Immunomodulatory activity has also been observed for a non-endotoxin like bacterial extract currently under chemical characterization. This adjuvant (denominated R 261) accelerates and increases the peak and the duration of the humoral response to protein and corpuscolated antigens after minimal and optimal stimulation, increases the bactericydal and cytotoxic activity of macrophages, determines an increase in DNA synthesis in the spleen. Effects can be seen after optimal, as well as minimal stimulatory challenges and the timing and schedule of treatment do not appear to be very critical for these effects. On the other hand no activity on the GVH has been observed and results compatible with enhancement of tumor growth obtained. Further studies on the possible selectivity of this agent on the various immunocyte sub-populations are underway.

Results of project No. 2

Investigators : A.Anaclerio, Ph.D. and F.Spreafico, M.D.

Title of project : STIMULATION BY IUdR OF T AND B SPLENOCYTES AND MACROPHAGES

IUdR (5-iodo-2'-deoxyuridine) is now used in the treatment of certain DNA virus infections. In contrast with the immunosuppressive activity generally shown by antimetabolites, IUdR was found to stimulate antibody production to allogeneic erythrocytes in mice. In the effort to understand its mechanism of action at the immunocyte population level, it was observed that IUdR increased total spleen cellularity doubling it with optimal immunostimulatory doses. This increase is due to a raise in the number of splenic B cells whereas no changes in the number of T lymphocytes and macrophages were detectable. For this effect a careful choice of dosage is required, but single active dosages have a prolonged effectiveness, which does not seem to be attributable to a mitogenic activity of the drug.

Spleen cells from IUdR-treated mice show heightened responsiveness in vitro to E.Coli lipopolysaccharide and to concanavallin A, i.e. to mitogens selectively active on B and T cells respectively. The rate of clearance of colloidal carbon as well as the in vitro erythrophagocytic capacity of isolated macrophages is also increased by treatment with this agent. Thus, IUdR is capable of functionally activating T, B cells and macrophages but only of increasing the number of B cells in the spleen possibly through an effect on cell differentiation.

Results of project No. 3

Investigators : F.Spreafico,M.D., A.Mantovani, M.D., A.Tagliabue,Ph.D.

Title of project : FURTHER STUDIES ON THE DIC-MEDIATED IMMUNOGENICITY CHANGES OF TUMOR CELLS.

Previous studies of this laboratory have shown that the in vivo treatment of tumor-bearing mice with antitumoral compounds can lead to an increase in the immunogenicity of the neoplastic cells so that when fully viable, immunogenically modified cells are transplanted into normal syngeneic secondary hosts, tumor rejection occurs as when allogeneic cancerous cells are employed. Among the various antitumorals investigated, DIC (NSC 45388), a frequently clinically used alkylating, was shown to be the most effective immunogenicity-modifying agent. Studies have been conducted in order to determine the minimal treatment conditions to obtain tumor cells with raised immunogenicity in the L1210 and LSTRA, DIC-resistant lymphoma models. In both systems it has been found that highly immunogenic tumor cells (HITC) can be obtained even after single DIC injections, though repeated doses are more effective. For observing HITC a single DIC dose was more effective when given during cell growth than in the lag phase, and for a fixed drug dose, the tumor population size had to be within defined limits to obtain the best degrees of immunogenicity increases. With Cyclophosphamide as transfroming agent, single treatment cycles were effective in transforming resistant neoplastic cells but single doses were ineffective.

The primary host bearing the more immunogenic tumor cells cannot reject them due to the concomitant immunodepression induced by the transforming antitumoral agent; therefore attempts at the restoration of immunologic responsiveness of these depressed hosts were performed through the transfer of syngeneic lymphocytes. The time and dose parameters for a successful restoration of immunosuppressed hosts with i.v. syngeneic splenocytes resulting in complete resistance against allogeneic tumor transplants have been worked out for Cyclophosphamide induced immunodepression, whereas in the case of DIC no success has yet obtained. The latter seems in fact to functionally inactivate immunocytes without creating a "vacuum" in the spleen such as to allow repopulation by the transferred cells.

Results of project No. 4

Investigators : F.Spreafico,M.D., A.Tagliabue, Ph.D., A.Mantovani,M.D.

Titel of project : SYNERGISM OF THE COMBINATION C.PARVUM AND ARMING SERUM

In previous studies on the mode of action of the immunomodulator Corynebacterium Parvum we have shown that this agent induced an especially prominent and long-lasting stimulation in the activity of the immunocyte subpopulation mediating Antibody-dependent Cellular Cytotoxicity (ADCC) whereas the humoral arm of this mechanism did not appear to be modified by C.parvum. These findings suggested that therapeutic synergism could be expected combining this agent with specific antileukemia ADCC-positive (arming) sera. It was observed that in mice bearing high numbers (10^6) of the syngeneic L1210 leukemia, the combined treatment produced cures in a very high proportion of animals and significantly longer lifespans in the eventually succumbing hosts in conditions where either treatment alone was totally ineffective even on much lower neoplastic burdens. Single injections of very limited quantities of arming serum devoid of complement-dependent cytotoxicity were employed and this treatment alone could prolong survival only when the leukemia inoculum was 10^2 cells. Clear evidence of similar synergism between C.parvum and specific arming serum have also been obtained in the LSTRA-leukemia system. No synergism was on the other hand observed when the same arming sera were combined with another immunomodulator (Levamisole) which does not modify ADCC cell-mediating activity whereas

stimulating cell-mediated cytotoxicity as C.parvum. In addition to shedding light on the in vivo role of ADCC in the control of neoplasm growth, these findings offer indications on the potential benefits to be acquired in tumor treatment by the rationalized combination of multiple immunotherapeutic approaches acting at complementary levels, a line heretofore not previously investigated.

Vertragspartner der Kommission:

Land Baden Württemberg, vertreten durch die Universität Ulm und mit Unterstützung durch das Bundesministerium für Forschung und Technologie sowie die Deutsche Forschungsgemeinschaft).

Nr. des Vertrages: 088-72-1 BIA D

Leiter der Forschungsgruppe:

Prof. Dr. Theodor M. Fliedner, Leiter der Abteilung für Klinische Physiologie der Universität Ulm und Sprecher des SFB 112 (Zellsystemphysiologie)

Allgemeines Thema des Vertrages:

Consequences of Radiation Exposure : Prevention and Treatment of Pathological Effects. - Evaluation of radiation injury, treatment of hematopoietic failure, basic research on cell systems, relevant to the evaluation and treatment of radiation injury in man.-

Allgemeine Darstellung der durchgeführten Arbeiten:

The research work of the Ulm group continued in 1975 to contribute to all three major lines of research which are to be advanced by this contract : that is, to the evaluation of radiation injury by means of hematological techniques, to the treatment of hematopoietic failure, as seen after acute and chronic exposure of man to ionizing radiation, and to the characterization of cell renewal systems, especially of their stem-cell pools, as the prerequisite for the advancement of evaluation and therapy of radiation injury. The research work in Ulm on these topics is closely related to and made possible only by virtue of the investigations within the frame of the Sonderforschungsbereich 112 (supported by the Deutsche Forschungsgemeinschaft) that are related to the basic principles of cellular renewal and its regulatory mechanisms. Part of the work carried out is being supported by the Bundesministerium für Forschung und Technologie.

Significant advances were made with respect to the evaluation of damage caused by ionizing radiation. Project No. 1 studied the ef-

fects of tritiated compounds on the hematopoietic tissue of rats during embryogenesis and in adulthood using biochemical and electronmicroscopical approaches. In addition, studies were initiated to explore the quantitative and qualitative determination of the stem-cells circulating in the peripheral blood with respect to their use as indicators of low-level radiation exposure. In an extensive preclinical pilot study, the daily fluctuations of blood stem-cells in normal dogs were studied to obtain background information for acute and chronic low-level exposure. Furthermore, the patterns of regeneration of granulocytically committed stem-cells (using the agar technique to demonstrate CFU_c) was studied in bone marrow and blood after irradiation and blood stem-cell transfusion. These studies will be transfered to the clinical level in the near future.

The second main topic of research "Treatment of hematopoietic failure" was in the center of interest of the Ulm group in 1975. The basic idea was to establish a preclinical model of a "blood stem-cell bank" for the treatment of hemopoietic failure after whole-body exposure to ionizing radiation. Mononuclear blood cells, collected by leukocytapheresis from beagles and cryopreserved over prolonged periods of time at -196° C, were given under autologous and allogeneic conditions. There is evidence for a graft in all dogs and a return of hemopoiesis to normal levels within a few months in the autologous situation. In the allogeneic dogs, only one dog survived for more than 2 years without immunosuppressive treatment. When immunosuppression was performed after blood leukocyte grafting, a large proportion of the dogs survived and showed hematopoietic restoration. A large segment of the work was carried out on the question of donor-recipient relationships using a variety of techniques to characterize the tissue by their immunogenetic markers. Studies are underway to reduce the graft-versus-host disease by additional histocompatibility markers, by separating stem-cells from immunoreactive lymphocytes and by gnotobiotic techniques. The supportive care of patients with hematopoietic failure - using leukemia as a clinical model - was studied extensively. The prospective study on the effect of bacterial decontamination of patients on the morbidity and mortality of infectious episodes in leukemic patients

was completed. Furthermore, the use of granulocytes, obtained by various methods, for transfusion into patients with hematopoietic failure,was investigated extensively.

The third area of research, "cell system research relevant to the evaluation and treatment of radiation injury", was also furthered markedly in 1975.It was of interest to study the interrelation of various stem-cell compartments and their interaction with leukemic cell proliferation in suitable animal models. Furthermore, the effect of radiomimetic substances, such as cycle-specific hydroxyurea, was investigated. In this case, it was of interest to follow the very early hematopoietic recovery by means of electronmicroscopical autoradiography in the bone marrow of animals in whom the entire latently resting cell population (cellular matrix and, presumably, some of the uncommitted stem-cells) was labeled. An attempt to characterize the uncommitted stem-cell population was also carried out in mice, to whom tritiated thymidine was given for several weeks during hemopoietic recovery after whole body x-irradiation and bone marrow transfusion. In collaboration with Prof. Lucarelli (Pesaro) a series of experiments was carried out to investigate fetal stem-cells in a variety of test systems with respect to their developmental potentialities.

Ergebnisse des Projektes Nr. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter:

T.M. Fliedner with W. Calvo, R.J. Haas, E.B. Harriss, M. Körbling, W. Nothdurft, W. Ross, W. Schreml, P. Szemere and K.H. Steinbach

Titel des Projektes:

Evaluation of radiation damage after external and internal radiation exposure.

Results:

In 1975, emphasis was placed on the study of tritiated compounds in rats during their development and in their adult life and on the evaluation of the effects of ionizing radiation on the stem-cell in blood and bone marrow with and without blood stem-cell transfusion.

The study of the effects of tritiated thymidine on the oocytes of rats during their intrauterine development was continued. It was found that the effects seen in new-born rats after continuous thymidine administration from day 9 of pregnancy until term are actually instituted during the 13^{th} - 15^{th} day, when the ovaries appear to be in their most radiosensitive phase. Administration of tritiated thymidine during days 9 - 12 reduces the oocyte number at birth only slightly, while the same dose given from days 13 - 15 reduces the oocyte number at birth by 50 % of normal. Tritiated water also has a marked effect, even if given only from day 9 to day 12, probaböy because the concentration of tritiated water remains high during the subsequent days of high oocyte radiation sensitivity. From all data obtained up to now which have been mentioned in previous reports, it is felt that the embryogenesis of the rat (or mouse) can be employed successfully as a model to compare the effects of radionuclides in various metabolic forms with those of external homogeneous whole-body irradiation.

In adult rats (180 - 200g), the radiotoxic effects of an 18-day continuous infusion of tritiated thymidine was studied, using bone marrow changes as an endpoint. Each animal received 864 µCi per day with a specific acitivity

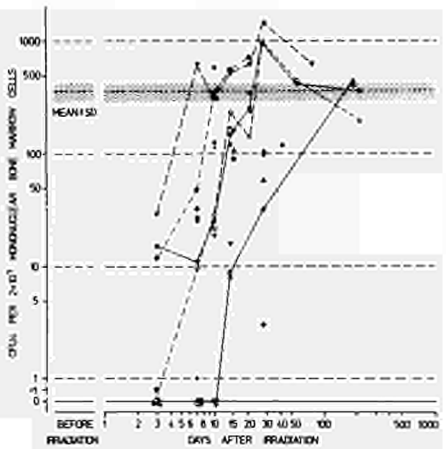
of 6 mCi/mMol. It is clear that tritiated thymidine will produce a distinctly inhomogeneous whole-body exposure because of its selective incorporation into DNA synthesizing cells. It thus will selectively eliminate "cells in cycle", while the latently resting cells in the bone marrow (for instance, endothelial, endosteal and reticular cells, as well as a small group of marrow lymphocytes) will not take it up and hence should provide the source for successful regeneration. It was of great interest to observe that the initial marrow destruction was associated with extravasation of red cells from the sinusoids into the parenchyma - as seen in whole-body x-irradiation. This may well indicate that early bone marrow hemorrhage is not so much due to primary injury of the vascular architecture per se, but is secondary to the elimination of the intraparenchymal growth pressure. This pressure is normally provided by the "cells in cycle" and compensated for by the flow pressure of the circulating blood so that there is usually no evidence of hemorrhage in the marrow. Various degrees of hypoplasia were observed but not all the parenchymal cells of the marrow were eliminated. Even in one of the animals that showed severe hypoplasia, small groups of erythroblasts and granulocyte precursors were still present in the endosteal area. The megakaryocytes appeared to be particularly resistent because they were numerous in all animals. In the endosteal area there were also numerous lymphocytes. It was of interest to note at the end of the infusion period, regeneration of the marrow parenchyma commenced immediately. This indicates that there were enough hemopoietic stem-cells left to initiate regeneration. It will be of great and particular interest to use this model with respect to the question, whether an irradiated reticular matrix is as good at supporting parenchymal regeneration as a relatively unexposed matrix, such as in this experiment.

The second aspect of this project concerns the use of blood stem-cells as an indicator of external and/or internal, homogeneous or inhomogeneous whole-body exposure. In the preclinical dog model, the daily fluctuations of the CFUc concentration in the blood were studied. It was found that there is a normal concentration of $94 \pm 72^{*} CFU_c$ per ml blood. There was a suspicion of a systematic oscillation with a wave length of 14 to 20 days. However, extensive statistical analysis could not support this notion in most of the dogs. Therefore, an attempt will be made to investigate the question

* mean $\pm$ S.D.

of blood leukocyte oscillations, especially of stem-cells, after the administration of radiomimetic drugs. The blood CFU_c concentration was also studied during repeated and extended extracorporeal irradiation of the blood. This approach will yield useful information on the exchange of blood stem-cells with extravascular sites. In dogs given 1200 R whole-body x-irradiation, the blood content of CFU_c decreased within 3 days to zero levels and remained so, unless a transfusion of fresh or cryopreserved blood mononuclear leukocytes (including pluripotential stem-cells) was given shortly after irradiation. In general, as seen in Fig. 1,

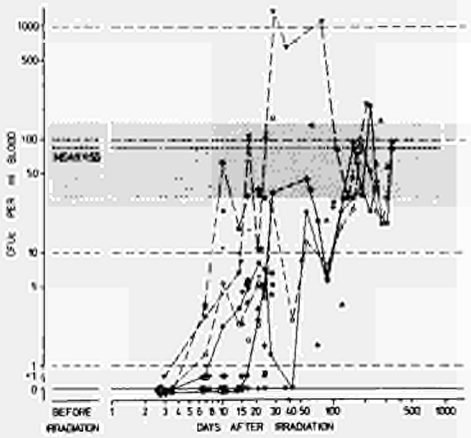
Fig. 1



the CFU_c concentration of the bone marrow approaches the normal range (357 ± 64* per 2×10^5 mononuclear cells) between 10 and 14 days after stem-cell transfusion; it made no difference whether $10 - 40 \times 10^9$ mononuclear autologous or allogeneic cells were given. At lower cell numbers, the return of the CFU_c concentration in the marrow was somewhat delayed. The CFU_c concentration in the blood returned to normal somewhat later (Fig. 2).

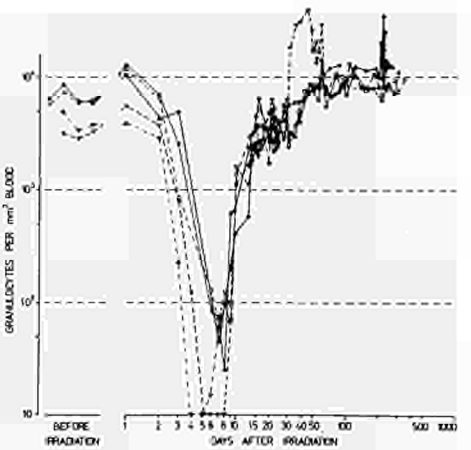
* mean $\pm$ S.D.

Fig. 2



Only a few showed a return to normal within 2 weeks. Most of the animals showed a return to normal between 30 and 120 days in parallel with the normalisation of the blood leukocytes in general (Fig. 3).

Fig. 3



It may well be that this slow return of CFU_c to normal reflects the slow reestablishment of the normal structure of the hemopoietic stem-cell pool and thus provides a possibility to evaluate the damage to bone-marrow and its repair in the most crucial compartment.

These results indicate that the concentration of CFU_c in the peripheral blood may well turn out not only to be a useful indicator of damage to the hemopoietic cell renewal systems, but also to reflect the true capability of the marrow to initiate and maintain a normal hemopoiesis. It can now be seen in what way the CFU_c content of the blood may be used as a prognostic indicator of hemopoietic activity.

Ergebnisse des Projektes Nr. 2

Leiter des Projektes und wissenschaftliche Mitarbeiter:

T.M. Fliedner, H.D. Flad, W. Nothdurft with C. Bruch, W. Calvo, S.F. Goldmann, E.B. Harriss, R.P. Huget, M. Körbling, K. Krumbacher, W.M. Ross and H.P. Schnappauf.

Titel des Projektes:

Blood stem-cell transfusion into lethally irradiated dogs as a preclinical model for the treatment of radiation-induced hemopoietic failure.

Results:

In 1975, the emphasis of the studies was on 4 areas. (1) Comparison of dogs given allogeneic blood mononuclear leukocyte transfusions (cryopreserved cells) that did or did not receive methotrexate as an immuno-suppressive agent. (2) Study of the pattern of regeneration of bone marrow and spleen after 1200 R whole-body x-irradiation and blood stem-cell transfusion. (3) Study of possibilities to mobilize blood stem-cells and (4) Study of the characterization of donor and recipient relationships by histocompatibility testing in dogs.

The first area concerns the question, whether dogs can survive a graft-versus-host reaction after having received a transfusion of cryopreserved blood mononuclear leukocytes subsequent to 1200 R whole-body x-irradiation. Table 1

Tab. 1

AUTOLOGOUS BLOOD LEUKOCYTE TRANSFUSIONS

(as of Aug. 20, 1975)

No.	Id. No.	No. trans. per kg b.w. x 10^9	"Take"	Survival (days)
1	77041	1.6	+	> 791
2	R 4	1.2	+	> 683
3	97071	1.5	+	> 523
4	67033	1.6	+	> 483

Tab. 2

ALLOGENEIC BLOOD LEUKOCYTE TRANSFUSION
(DLA identical, MLC negative; no immunosuppression)
(Status Aug. 20, 1975)

No.	Id. No.	No. cells p. kg b.w. x 10^9	"Take"	Chim. Chrom.	GVHR Sk. + MM	GVHR Bl. Ch.	GVHR Path.	Surv. (days)	Remarks
1	67039	0.5	+	-	-	-	o	14	
2	92127	1.1	+	-	+	++	+++	15	
3	92129	1.1	+	KM;	o	o	o	8	(1)
4	97073	1.2	+	-	o	o	o	8	
5	67038	1.3	+	-	o	o	o	6	
6	127075	1.3	+	KM; -	++	++	+++	27	
7	67029	1.5	+	KM; PB	++	+	+++	66	
8	57017	1.5	+	KM; PB	(+)	++		>791	
9	97072	1.7	+	-	(+)	++	o	20	(2)
10	67030	2.8	+	KM;	++	(+)	+++	16	
11	113115	0.6	+	KM; -	+	(+)	-	19	
12	113117	0.6	+	KM; -	++	o	+++	21	

(1) Only G 1 damage, in liver and skin no gvh reaction
(2) Multiple abscesses in liver and spleen (fungi; E. Coli; Strept. faecalis)

Tab. 3

ALLOGENEIC BLOOD LEUKOCYTE TRANSFUSION
(DLA identical, MLC negative; with Methotrexate treatment - 100 d)
(Status Aug. 20, 1975)

No.	Id. No.	No. cells p.kg b.w. x 10^9	"Take"	Chim. Chrom.	GVHR Sk. + MM	GVHR Bl. Ch.	GVHR Path.	Surv. (days)	Remarks
1	2232	0.6	+	KM; PB	(+)	+		> 287	
2	138	0.6	+	KM; PB	o	++	o	261	(1)
3	129	0.6	+	-	+	(+)	-	26	
4	1211	0.7	+	KM; -	+	++	+	45	
5	1212	0.8	+	KM; PB	++	+		> 310	
6	139	0.9	+	KM; PB	(+)	(+)		> 310	
7	61102	1.5	+	-	+	+	-	25	
8	77043	1.9	+	-	+	(+)	+++	23	
9	61114	0.7	+	-	o	(+)		> 84	
10	113113	0.7	+	KM; -	++	++	++	29	(2)
11	113112	0.7	+	KM; -	o	++		> 84	
12	82106	0.5	+	-	o	o	o	8	(3)
13	113119	1.0	+	KM; -	++	+	+++	27	
14	113116	0.9	+	-; PB	+	++		>182	

(1) Viral infection (Liver: cytomegalic incl. disease, ascites)
(2) Bronchopneum.
(3) Intusception of jejunum

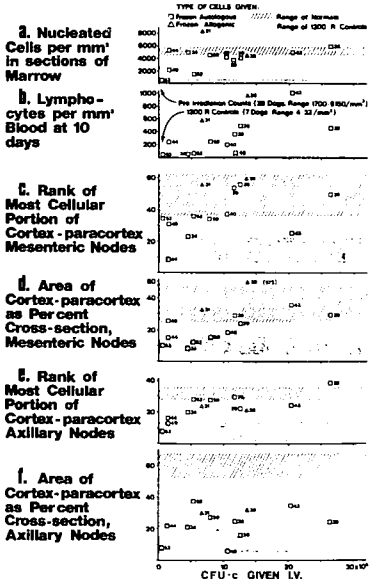
shows the results of autologous transfusions as of Aug. 20, 1975, Table 2, those of allogeneic transfusions without methotrexate, and Table 3, those of allogeneic transfusions with methotrexate as an immunosuppressive agent.

It can be seen (Table 1) that we have now been following 4 dogs for 1 - 2 years after transfusion of cryopreserved autologous blood leukocytes. This indicates to us, that stem-cells collected from blood in dogs are as good at initiating and maintaining hemopoietic recovery as stem-cells from bone-marrow (as reported in the literature). - Of 12 beagles given between 0.5 and 2.8 x 10^9 allogeneic mononuclear blood cells, but not subjected to immunosuppression, all showed evidence for a "take" but only one survived for more than 2 years as a chimera (Table 2). All other animals died early (from infection and/or bleeding) or at a time of severe graft-versus-host disease. If methotrexate was used (Table 3) the survival was markedly improved. These studies indicate also that there is no real difference in the efficiency of equal numbers of autologous and of allogeneic blood mononuclear leukocytes to initiate hemopoietic regeneration (unlike the situation for bone marrow!) and that the severe contamination of the blood cells with lymphocytes does not appear to produce more serious gvh-complications than one would expect after a bone marrow transfusion. The establishment of long-term chimeras can be improved by the use of immunosuppressive agents, such as methotrexate.

Furthermore, this study provided the opportunity to study the pattern of regeneration of cell production in bone marrow and lymphatic organs and the return of blood cells to normal. Some results have been reported in previous years. In 1975, the early changes (10 days after x-irradiation and transfusion) in the spleen and lymphnodes were investigated thoroughly. It is clear from these studies that the transfused cells contained elements capable of restoring a great deal of lymphopoiesis in spleen and lymphnodes within 10 days. It remains to be determined to what extent there is a correlation between the number of CFU_c transfused or the number of immuno-reactive lymphocytes transfused and the regeneration of the lymphatic organs.

The most important findings are demonstrated in Fig. 3 which shows the relationship of the number of CFU_c transfused and the regeneration of cell production in lymphatic organs.

Fig. 3



The third aspect of our studies in this project was concerned with the possibility of mobilizing mononuclear cells and CFU_c by heparinoids, sulphate-containing polyanions with strong anticoagulant action. The increase in mononuclear cells begins within the first hour after intravenous injection of the polyanion, reaching a peak value after about 3 hours of 3 - 4times normal and then declining over the subsequent 5 - 6 hours to control values. Dextran sulphate and polyvinyl sulphuric acid are also capable of mobilizing hemopoietic stem cells; the former is apparently the better and no toxic effects are observed. The increase in CFUc in the peripheral blood of dogs begins very soon after injection, reaching a peak of 6 - 10times normal at about the same time as the peak for mononuclear cells. However, the mobilisation of these two cell populations (CFU_c being part of the mononuclear cell population) is not in synchrony. In dogs, the mononuclear cells remain at a high level for some time, while the CFU_c begin to decrease immediately; this is especially noticeable with high doses of dextran sulphate (15 mg/kg).

The main reasons for trying to mobilize stem cells into the peripheral blood are :

a, to increase the number of CFUc (stem cells) collected during continuous flow centrifugation for transplantation;

b, to increase the number of stem cells available for killing with procedures such as extracorporeal irradiation of blood to study stem-cell migration between bone marrow sites.

Results to date are promising. After 10 mg/kg dextran sulphate in one dog, a 5-hour leukocytapheresis using the IBM blood cell separator yielded 16 x 10^5 stem cells. After 15 mg/kg in another dog, which already had a high CFUc count in blood at time 0 (10,000/ml; probably a result of previous dosages of dextran sulphate), a 3-hour centrifugation yielded 50 x 10^5 CFUc stem-cells. In a regeneration experiment, several dogs were given different amounts of mononuclear cells with varying proportion of stem-cells (CFUc) and sacrificed on day 10. The stem-cell concentration ranged from 0.3 - 26.3 x 10^5, usually from 2 - 3 five-hour leukocytaphereses.

Finally, progress was achieved with the histocompatibility matching in dogs as a prerequisite of allogeneic blood stem-cell transfusion. The mixed lymphocyte culture system (MLC) was adapted for this purpose. In several mammalian species, a chromosomal region has been recognized with a number of closely linked genetic systems which have a major influence on histocompatibility. Recent reports of MLC data in man and mice have clearly established that the MLC reactivity is genetically determined by one or more loci closely linked to but separate from the serologically defined (SD) HLA and H2 loci, respectively. Preliminary evidence for the existence of a separate lymphocyte defined (LD) MLC locus (or loci) in the DLA region has been provided in a few experiments.

Dogs are used as experimental models for allogeneic stem-cell transfusion. The MLC test seems to measure effective transplantation determinants not detected so far by serological means. After having established a reliable and reproducible MLC test in dogs (Goldmann and Flad 1975), we started to study the correlation between MLC reactivity and DLA-SD haplotypes in related dogs of different kennels.

Lymphocytes of kennel members with DLA-SD identical haplotypes did not stimulate each other in the MLC. This implies that LD typing in related dogs can be generally predicted by DLA-SD typing. Consequently, lymphocytes of related dogs homozygous for a given DLA-SD haplotype could be shown, with few exceptions, to be also homozygous for MLC determinants (Table 4). Six different MLC determinants were defined in DLA-SD and LD homozygous dogs (Table 5). Three additional MLC specificities could be recognized on DLA-SD and LD heterozygous cells (Goldmann et al. 1975).

It will be the next step to study the separation of hemopoietic stem-cells from the bulk of mononuclear blood leukocytes and to see the effects of their transfusion on the regeneration of bone marrow and lymphatic organs and on the extent and severity of gvh.

Tab. 4

Matching for LD using heterozygous and homozygous cells

				STIMULATING CELLS (x)			
		Dog No.	DL-A SD	Ax	Bx	Cx	Dx
RESPONDING CELLS	A	73/29	9,4/7,-	872	22,988	25,080	432
	B	73/9	9,4/3,-	35,518	191	34,886	334
	C	65/1	9,4/2,5	8,570	4,605	365	506
	D	73/25	9,4/9,4	19,984	26,966	31,816	405

Results are given as mean CPM of triplicate cultures

Tab. 5

Reference cells (dogs) for LD typing

	DL-A LD	DL-A SD	Breed	Dog No.
1	I/I	2,4 / 2,4	Beagle	2228, 2231
2	II/II	2,5 / 2,5	Beagle	67026, 67028
3	III/III	9,6 / 9,6	Beagle	111
4	IV/IV	9,4 / 9,4	Foxhound	73/22
5	V/V	7,13/ 7,13	Pointer	48, 64
6	VI/VI	1,13/ 1,13	Beagle	3249

Ergebnisse des Projektes Nr. 3

Leiter des Projektes und wissenschaftliche Mitarbeiter:

T.M. Fliedner, M. Dietrich (clinical), H. Heit (experimental) with C. Abt, W. Heit, D. Krieger, H. Meyer, H. Pflieger, H. Rasche, E. Vanek

Titel des Projektes:

Bacterial decontamination as a means of combating bacterial infection and graft-versus-host disease in patients and experimental animals with bone marrow failure and after bone marrow transfusion.

Results:

The EORTC-Gnotobiotic Project Group has completed in 1975 the analysis of its prospective study which had the goal to examine and compare the use of a protected environment (together with or without bacterial decontamination by means of antibiotics) with treatment in the open ward. This collaborative European study was coordinated and evaluated by the Data Center of the University of Ulm. From this study, the data of 137 patients with hemopoietic failure due to acute myelocytic leukemia could be analysed. The data show that - using the induction of remission as an end-point - the treatment inside a protected environment (remission rate 69 and 61 %) is superior to the treatment in an open ward (remission rate 49 %). The study demonstrated further that the median survival for patients in a protective environment with the administration of antibiotics is 245 days, while it is 222 days for patients in an isolation system but with no systematic decontamination. The median survival in an open ward was 177 days. It is clear from this study that emphasis must now be given to the exploration of ways and means to eliminate even more bacteria than was possible until now. This may well require new approaches with respect to the regimens of decontamination previously used.

Experimental Gnotobiology:

In the last report, it was demonstrated in mice that bacterial decontamination and the maintenance of animals in a germfree environment have a pro-

found effect on the gvh-mortality after allogeneic bone marrow grafting. It was shown that a stable chimerism of marrow cells from the donor was established in the recipient. The re-conventionalization of the gnotobiotic animals after 100 days was tolerated very well. None of the animals died.

In 1975, a study was performed on the ability of the chimeric bone marrow to protect in second passage, radiation-induced aplastic recipients and to induce gvh-R in the recipients. Bone marrow suspensions from 40 week old allogeneic chimeras (CBA/CA mice, grafted with $C_{57}Bl$ bone marrow) were grafted into conventional, lethally irradiated CBA/CA or $C_{57}Bl$ mice. To enhance graft-versus-host reaction in the recipients, 2 x 10^6 spleen cells from the chimeras were added and injected together with the bone marrow cells. The results are summarized in Table 5.

Tab. 5 SURVIVAL OF CONVENTIONAL IRRADIATED MICE GRAFTED WITH HEMOPOIETIC TISSUE FROM ALLOGENEIC DONORS OR LONG TERM BONE MARROW CHIMERAS*

a)

DONOR MATERIAL			RECIPIENTS		100% SURVIVAL
STRAIN	TISSUE	CELL DOSE GRAFTED	STRAIN	NO.	(WEEKS)
C57Bl-CBA (CHIMERA)	B.MARROW	1 x 10^8 i.v.	C57Bl	10	>16
C57Bl-CBA	B.MARROW	1 x 10^8 i.v.	CBA	10	>16
C57Bl-CBA	B.MARROW SPLEEN	0,8 x 10^8 i.v 2 x 10^8 i.p.	C57Bl	10	>16
C57Bl-CBA	B.MARROW SPLEEN	8 x 10^8 i.v 2 x 10^8 i.p.	CBA	10	>16

*40 WEEKS AFTER ALLOGENEIC B.M. GRAFTING

b)

DONOR MATERIAL			RECIPIENTS		25% SURVIVAL
DONOR	TISSUE	CELL DOSE GRAFTED	STRAIN	NO.	(DAYS)
C57 Bl	B.MARROW	10 x 10^6 i.v.	CBA	20	32
C57 Bl	B.MARROW SPLEEN CELLS	10 x 10^6 i.v. 2 x 10^8 i.p.	CBA	20	17

Compared to primary allografting, the same bone marrow dose of 10 x 10^6 cells was found to be sufficient to protect lethally irradiated allogeneic recipients, while in the isogeneic situation 1 x 10^6 bone marrow cells were as effective in providing comparable protection against postirradiation mortality. No signs of secondary disease were observed in any of the ex-

perimental groups (Table 5a). Even those chimeras which were injected with both bone marrow and spleen cell suspensions did not reveal increased mortality or signs of secondary disease; this was in opposition to conventional allogeneic recipients transplanted with identical doses of cells of primary donor origin (Table 5b). Thus, we tentatively conclude that in both the bone marrow and the spleen of long-term chimeras, the lymphocytic population may lose its potentiality to react on the same type of histocompatible antigens encountered in the primary host. Since no blocking effect of chimeric sera in cell-mediated lympholysis was observed at any phase of the experiments, humoral "inactivation", which has been discussed by Hellström et al., seems to be of little relevance.

Ergebnisse des Projektes Nr. 4

Leiter des Projektes und wissenschaftliche Mitarbeiter:

T.M. Fliedner, W. Calvo, E.B. Harriss, D. Hoelzer

Titel des Projektes:

Studies on the function of the hemopoietic stem-cell pool after radiomimetic substances and during interaction with a leukemic cell population

Results:

The emphasis of the experimental stem-cell studies in 1975 was on the attempt to characterize - by means of semi-thin sections and electronmicroscopy - the cellular pattern of bone marrow in rats after hydroxyurea. The animals used were labeled with tritiated thymidine in "resting" bone marrow cells. This ^{3}HTdR labeling had been achieved by infusing it continuously during pregnancy; the labeling was maintained for 4 weeks after birth by serial ^{3}HTdR injections ($\frac{}{q}$ 8 hours). In order to recognize only resting cells, no label was given between 4 and 8 weeks after birth. This allowed all cells actively turning-over to discharge their label. Only endothelial cells, reticular cells and a small fraction of bone marrow lymphocytes remained labeled. It was felt and shown that these labeled, resting lymphocytes were involved in the initiation of regeneration, but not the reticular or endothelial cells. An aplasia of the marrow cells was achieved by administration of 4 x 500 mg of hydroxyurea/kg (HU) at 6 hr intervals. The regeneration was studied afterwards by electronmicroscopy and in methacrylate-embedded tissue by means of autoradiography. It was found that, at the end of the month without thymidine, all the stroma cells were marked in the bone and in the marrow. In addition, some mononuclear cells with fine structure indistinguishable from that of lymphocytes, were also marked. The administration of HU destroyed the parenchymal cells within 24 hours, while the lymphocytes and the stromal cells of the BM and the cells of the bone remained. These cells retained their label for the duration of the experiment (7 days after administration of HU). Numerous lymphocytes, some of them still labelled, were present at every stage of the experiment. On the 3rd day after HU they were numerous in the endosteal area. On the 4th day,

groups of granulocyte and erythrocyte precursors were also seen, some of them weakly marked. On the 7^{th} day, non-labeled granulocytes were numerous while reticulum cells and endothelial cells remained heavily marked. This fact indicates that stroma cells do not give rise to the cells of the marrow parenchyma but severe only as supportive elements. Streams of marked osteoblasts and osteocytes invaded the bone cavity from the 3^{rd} day on after injection of HU and marked osteoclasts became large and numerous between the marrow and the bone trabeculae. These studies gave us the opportunity to study the origin of cellular regeneration in a hydroxyurea-induced bone marrow aplasia and indicated the electronmicroscopial features of the mononuclear cell involved in the restoration of hematopoietic parenchyma.

A second series of experiments was concerned with normal and leukemic stem-cells. During experiments relating to the involvement of hemopoietic stem-cells in recovery of normal hemopoiesis after chemotherapy for an experimental leukemia (L5222) in rats, it was observed that the cytostatic agent, Daunorubicin, had less effect on the normal granulopoietic progenitor cells (CFU_c, as tested in the agar colony technique) in leukemic rats than on those in normal rats. A possible explanation for this could be that, owing to an inhibiting influence of leukemic cells, a smaller proportion of normal hemopoietic stem-cells are in DNA-synthesis in leukemic than in normal rats. This hypothesis was tested using the ^{3}H-thymidine suicide technique on cell suspensions in vitro and it was found that an average kill of CFU_c of only 25 % was achieved in leukemic bone marrow compared with 40 % in normal bone marrow. Comparative in vivo experiments on the effects of hydroxyurea (a further agent which affects mainly cells in the S-phase) on CFU_c in normal and leukemic rats are in progress.

Ergebnisse des Projektes Nr. 5

Leiter des Projektes und wissenschaftliche Mitarbeiter:

G. Lucarelli with A. Porcellini, T. Izzi, M. Galimberti, M. Tomasucci, A. Fontebuoni, A. Bravetti and E. Guardato

Titel des Projektes:

Comparative study on the characterization of the fetal and neonatal stem-cell compartments as a basis for studies on the regulation of hemopoietic cell kinetics.

Results:

Since 1973 studies were performed on the "characterization of fetal and neonatal stem-cell pools". The purpose of the original experimental design was to acquire information on the fetal hemopoietic tissue as a potential source of hemopoietic stem-cells for bone marrow transplantation in human bone marrow aplasia. From previous studies it appeared that the fetal hemopoietic stem-cell could only partially respond to the stimulatory effect of a humoral factor known to exert an effect on the stem-cells of adult animals, encouraging differentiation toward the erythroid line. These studies suggested extension of the observations to the newborn rat at different ages in order to obtain information on the regulatory mechanism of erythroid differentiation. The extensive experimental design was reported in "Scientific Report 1973-1974". In the 1975 experimental program the maximum effort has been directed towards the study of the developmental phases of fetal hemopoiesis; fetal liver was cultured in diffusion chambers implanted intraperitoneally into irradiated mice.Parallel studies have been initiated on the effect of testosterone on bone marrow cells as a substance to be extensively used in stimulating the proliferation of transplanted fetal hemopoietic tissue. - Differentiation of fetal hepatic tissue in an "in vivo" culture system : Its response to endogenous erythropoietin in 16-day-old mouse fetal liver suspensions cultured in diffusion chambers implanted in normal non-irradiated host mice as well as in anemic mice. Both groups showed a marked decrease of erythroid elements parallel to the growth of granulocytic elements. Such a decrease was faster in the

group of cultures implanted in bled mice. The effect of the higher erythropoietin level on cultured fetal liver cells could be an acceleration of hemoglobin-synthesis and reduction of transit-time within the maturation compartment of erythroblasts. This effect could be separated by the ability of erythropoietin to stimulate erythroid committed stem-cells to mature (as demonstrated in vivo and in vitro by many) because the diffusion chamber-microenvironment is not able to sustain erythropoiesis like it does granulo- and macrophagopoiesis. Effect of testosterone on hemopoiesis in diffusion chamber cultures: The erythropoietic action of testosterone is probably due to a direct effect on erythropoietin production; as recently demonstrated by others, this steroid hormone acts on the committed stem-cell. From our data, the effect of testosterone on the stem-cell (as measured by the method of Till and McCulloch) was evident. When we cultured mouse bone marrow in diffusion chambers in testosterone-treated host mice, better growth was observed, relative to bone marrow cultures in normal host mice. This cellular increase was due to increased production of granulocytes and macrophages. In treated-mice cultures, the number of CFUs/DC was also increased. Our data suggested that testosterone is able to act at all levels of the maturation of hemopoietic cells. Studies have been performed using the CFU spleen-technique in order to evaluate the bone marrow content of stem-cells of different ages in the newborn rat. From preliminary data it appears of great interest that rat-to-rat bone marrow transplantation (in order to obtain CFUs) is possible when a 21-day-old rat is used as irradiated recipient and sacrified at 30 days of age. The CFUs technique was practically of no use when adult rats were used, as compared with the classical method in mice. This study is under development and will be completed in 1976.

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Contractant van de Commissie	: Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek TNO
Nummer van het contract	: 088-72-1-BIAC
Hoofd van de researchteams	: Prof. D.W. van Bekkum
Algemeen onderwerp van het contract	: Consequences of radiation exposure, prevention and treatment of pathological effects

General description of the program

Histocompatibility typing continues to dominate the search for selection of suitable bone marrow donors in both the monkey and the dog model.

The methods of typing and matching for the various categories of tissue antigens controlled by the MHC*, are being improved in Rhesus monkeys.

- SD antigens. About 90% of the gene products of the two SD loci can now be reliably identified (the conventional "transplantation antigens"). Transplantation experiments in which unrelated donor/recipient pairs shared the complete set of 4 SD antigens ("full-house identicals"), confirmed earlier impressions that skin allograft survival is significantly prolonged by SD-matching. Experiments still in progress suggest that this is not the case for kidney allografts exchanged between similarly matched host/donor combinations. Transplantations with bone marrow from full-house identical, unrelated individuals have not yet been performed (see plans for 1976).
- LD antigens. The determination of these important cellular markers was approached from several angles:
  - "LD typing cells" : this method makes use of RhL-A homozygous cells which permit the recognition of the stimulator antigens of the major MLC locus. Disadvantages are the inevitable use of the whimsical MLC test and the limited availability of LD typing cells.
  - the PLT or Primed Lymphocyte Test : This method which was recently introduced by Bach and colleagues in Madison, USA, also seems to work in monkeys; the advantage is that determinations of LD antigens (with frozen PLT cells) can be performed within 24 hours. However, numerous technical problems remain to be solved before this method will be a reliable test procedure in LD typing of monkeys, man or rodents.

- serological identification of LD determinants is not yet feasible in monkeys. So far, only one research laboratory (van Rood's in Leiden) seems to be successful in the serological identification of LD determinants. Previous attempts to raise anti-LD sera in monkeys, have led to the discovery of the la-like antigens described below.
- la-like antigens : the main progress in tissue typing of rhesus monkeys during 1975 was the identification of the so-called la-like antigens. 10 or 11 provisional specificities can be serologically determined (primarily on B-lymphocytes, not on platelets = "restricted tissue distribution"). In rodents, similar B cell specific antibodies have been shown to have "enhancing" properties in transplantation experiments and the identified la antigens of mice seem to be relevant to histocompatibility, particularly to GvH reactions. However, as indicated before, typing for la-like antigens in monkeys has not yet reached the stage to permit reliable matching experiments which might prove the relevance of la-like antigens to histocompatibility.

In dogs the effectiveness of currently available methods of donor selection in the prevention of severe GvH reactions in recipients of allogeneic bone marrow of related or unrelated donors were investigated. LD (lymphocyte defined) and SD (serologically defined) determinants of the major histocompatibility complex were studies in this regard.

Parallel with this development of effective matching procedures, investigations are being continued to improve current methods of selective elimination of immune competent cells from the bone marrow before grafting by physical (gradient) or immunological (ALS) means. In addition, the development of improved methods of support of the transplant recipient i.e. bacteriological decontamination and thrombocyte preservation are being developed.

Resultaten van het projekt No. 1

Hoofd van het team en wetenschappelijke medewerkers :

D.W. van Bekkum, H. Balner, P.J. Heidt, W.D.H. Hendriks,

B. Löwenberg, H.M. Vriesendorp, G. Wagemaker, W. van Vreeswijk.

Titel van het projekt : Bone marrow transplantation

Results in 1975

- 1. Total decontamination of monkeys prior to whole body irradiation and bone marrow stem cell grafting, has been continued. Unexpectedly, these experiments were complicated by early mortality which is unrelated to GvH disease. The cause of this early mortality is being investigated but so far an acceptable explanation cannot be provided. This part of the program is being severely delayed by this complication. In the meantime, investigations in the mouse model are being pursued to identify the bacterial species which are responsible for delayed GvH mortality in conventional animals. Tissue typing in monkeys was pursued with the objective of combining in the future the principle of donor selection with those of stem cell purification and stem cell decontamination. The development of matching procedures is expected to result in practical applications in the bone marrow transplantation in 1976.

2. In dogs LD and SD matching appeared to have a pronounced influence on the severity of GvH reactions in canine sibling donor-recipient pairs. No difference was found between the survival times of recipients of 1 or 2 MHC haplotype different bone marrow in the same model. In preliminary results LD or SD matching did not appear to influence the severity of GvH disease in unrelated donor-recipient pairs. This suggests the presence of other genetic loci with an influence on GvH reactions besides the currently known LD and SD loci. Donor selection studies will be continued in dogs as well as in monkeys. In the latter species la or "immune response associated" antigens can be determined and the influence of these structures on GvH reactions will be investigated.

The identification of genetic markers which control the occurrence of GvH disease in DL-A identical sibs has been unsuccessful so far in the dog model. New techniques will be applied to this problem such as "secondary"

mixed lymphocyte cultures and the use of stimulating cells other than lymphocytes in this technique. The optimal conditions for separating dog bone marrow cells on discontinuous albumen gradients in order to prepare concentrated cell populations of hemopoietic stem cells are being defined. Cyropreservation of dog bone marrow is also evaluated in a quantitative way to enable the use and evaluation of gradient purified bone marrow suspensions in this species.

3. The selective elimination of immune competent cells from bone marrow by incubation with ALG and complement in vitro has focused on the applicability of the in vitro colony formation as a monitor for stem cells. All ALS preparations exhibit a certain cytotoxicity towards stem cells and therefore conditions of incubation have to be worked out for each ALG preparation which permit maximum inactivation of immune competent cells and - at the same time - minimal inactivation of stem cells. In experiments with mouse bone marrow the results with spleen colony formation and in vitro colony formation did not run parallel, which renders it difficult to pursue this type of work with monkey and eventually human bone marrow. Since higher concentrations of ALG were found to stimulate in vitro colony formation, further purification of ALG is being performed for use in these experiments.

4. Attempts to identify culture conditions and factors which allow the multiplication of stem cells in vitro have been continued with bone marrow stem cells because these are easier to procure than fetal stem cells. Factors which stimulate the proliferation of stem cells are produced by fetal fibroblast cultures and by short time lymphocyte cultures following stimulation with PHA. Such stimulation cannot be provided by colony stimulating factor (CSF) nor by post-endotoxin serum. Attempts to concentrate and purify the stem cell stimulating factor(s) are being made but the instability of the activity so far provides technical problems.

5. The development of a method for cryopreservation of thrombocytes was completed in rhesus monkeys. Survival time in vivo of ^{51}Cr labelled cryopreserved thrombocytes (cooperation with Dept. of Immunohaematology, Leiden) did not differ significantly from freshly labelled thrombocytes. Experiments with lethally irradiated monkeys demonstrated a normal restoration of hemostatic function by cryopreserved thrombocytes. Starting January 1, 1976, a bank of cryopreserved thrombocytes will be available for routine use to support bone marrow transplantation in rhesus monkeys.

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Contractant de la Commission : Prof.H.J.TAGNON

N° du contrat : 088 BIAC

Chef du groupe de recherche : P.STRYCKMANS

Thème général du contrat : Kinetics and regulation of proliferation of normal and pathological bone marrow cells.

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1. Study of the factors determining normal and leukemic bone marrow cell proliferation and cell death.

A. The total body myeloid cell mass is obviously considerably increased in human chronic myeloid leukemia. However, this mass can be repeatedly reduced by intermittent chemotherapy. It is possible therefore to follow the proliferative activity of the myeloid precursors during therapy induced oscillations of the myeloid cell mass and to look whether an inverse correlation exists between the cell mass and the proliferation rate. The demonstration of such a correlation in leukemic patients indicates the persistance of a regulatory mechanism operating on leukemic cells (5, 6). A study was then undertaken to determine whether the regulation seen in leukemia is quantitatively equal to the one seen in non-leukemic individuals. This regulation was found to be decreased in chronic myeloid leukemia (7).

B. The effect of Vincristine, a drug known to inhibit the mitotic spindle formation, was investigated to see whether it is dependent on the proliferative activity of the cells. It was found that the DNA synthesizing cells are differently affected by this drug depending on their rate of DNA synthesis. This may be important when cell spindle poisons and DNA synthesis inhibitor agents are associated for instance in the treatment of leukemia.

2. Kinetic, morphologic and functional characterization of leukemic cells.

The cell responsible for human "hairy cell leukemia" was isolated and could be identified as a monoclonal B lymphoid cell, a question which was hitherto controversial. The recognition of the monoclonal production of this peculiar cell could lead to the identification of a new normal lymphocyte subclass.

3. The effect of granulocyte transfusions in infected neutropenic patients with bone marrow aplasia.

A randomized study was described in the 1974 report of activity. In the last year an attempt was made to define properly the indications for granulocyte tranfusions (1, 2). It appears that more randomized clinical studies are necessary to evaluate which infectious conditions, antibiotherapy resistance situations and neutropenic levels, are the best indications to transfuse polymorphonuclear cells.

Résultats du projet n°1

Chef du projet et collaborateurs scientifiques : P.STRYCKMANS, L.DEBUSSCHER, E.RONGE-COLLARD, D.GANGJI.

Titre du projet : Study of the sensitivity of proliferating bone marrow cells to regulatory mechanisms and external chemical agents.

A. Regulatory mechanisms

Our group has previously shown that in human leukemia at least in the chronic myeloid leukemia, the earliest recognizable myeloid leukemic cells namely the myeloblast is sensitive to a regulatory mechanism probably operating on normal myeloblasts (5). The proliferative activity of the myeloblasts in the bone marrow of patients with CML was found to be decreased in the active phase of the disease when the white blood cell (WBC) count was higher than 40, 000/cu mm. On the other hand, during remission of the disease, when the WBC was normal or less than 20,000/cu mm, the ^{3}H-thymidine labeling index of the marrow myeloblasts returned to a normal value indicating that these cells resumed a normal proliferative activity.

This observation of the persistance of a regulatory mechanism operating on the early myeloid cells in CML did raise the question of whether this regulatory mechanisms is set at a normal level in leukemia (6). In order to test this possibility, the marrow myeloblasts of CML patients were compared to those of non-leukemic patients. This comparison was made at different WBC levels. To enter the study, the non-leukemic patients with high leucocyte counts had to have this anomaly established for more than 2 weeks. It was found that non-leukemic patients with a WBC between 10 and 40,000/cu mm had a mean ^{3}H-thymidine labeling index of their marrow myeloblasts of 30.5 % i.e. significantly lower than the 49.9 % found in 9 hematologically normal individuals. This contrasts with the absence of significant difference between the labeling indexes of 5 CML patients with less than 10,000 WBC/cu mm (43 %) and 5 CML patients with 10-40,000 WBC/cu mm (46 %).

It is concluded that, although some regulation of myelopoiesis persists in CML, this negative feedback regulation is set at an abnormal level : in CML, it is put into action, only when the WBC reaches high levels which

are, in fact, rarely reached in non-leukemic conditions.

B. Chemotherapy

The mode of action of Vincristine (VCR) an alkaloid extracted from Vinca Rosea and very active against acute leukemia, on human bone marrow cells in vivo.

The drug was administered intravenously at the usual therapeutic dose of 2 mgr/m2 body surface area to 3 hematologically normal patients. In order to study the effect of this drug on the cell cycle, tritiated thymidine was injected to the patients just before VCR administration and specimens of bone marrow for autoradiographic and microdensitometric studies were taken 1 and 24 hours later. The results of this study are the following :

1. The metaphase blocking effect of VCR on the erythroblasts of the bone marrow is observed in vivo at least for 24 hours.
2. The cells in the DNA synthesis when VCR is injected are not equally sensitive to its action : those synthesizing DNA at a low rate at the time of VCR injection are preferentially seen in mitosis 24 hours later.

It is concluded that their rate of DNA synthesis is playing a role in the sensitivity of the erythroblast to VCR. It is not yet clear however whether a low rate of DNA synthesis makes the cell more susceptible to the blocking effect of VCR in mitosis or on the contrary less sensitive to the cytocidal effect of VCR in other phases than mitosis. The rate of DNA synthesis being higher in the more immature myeloid and erythroid cells it is possible that VCR will affect differently the less and the highly differentiated bone marrow cells.

Résultats du projet n°2

Chef du projet et collaborateurs scientifiques : P.STRYCKMANS L.DEBUSSCHER, E.RONGE-COLLARD, D.GANGJI.

Titre du projet : Characterization of the cell responsable for a special type of human leukemia called hairy cell leukemia.

Hairy cell leukemia, untill recently a rare type of human chronic leukemia, is now more and more diagnozed.
The nature of the hairy cell (HC) has been for long the subject of controversy. First considered as a primitive reticulum cell it was recently considered by some to have a lymphocytic origin and by others a monocyte-histiocytic origin.

An extensive analysis of the cells of one patient with hairy cell leukemia was performed (4) and showed :

1. The capacity of these HC to adhere firmly to plastic making it possible to obtain a pure population of HC.
2. Neither T-rosette formation nor phytohemogglutinin transformation with these HC.
3. Immunoglobulins on the surface of HC by immunofluorescence.
4. Synthesis and secretion by these cells of IgM type λ-clain by radioimmuno-diffusion.
5. A half life in the blood in vivo of $\sim$150 hours, similar to what is seen for CLL lymphocytes and very different from what is seen for normal monocytes.

It is concluded that hairy cell leukemia is a monoclonal disease resulting from the proliferation of a B lymphocyte. The peculiar aspect of the hairy cells could represent either an unusual morphologic expression of the neoplastic process or the expression of a special function of a lymphocyte subclass or differentiation stage.

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Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung
mbH München
Institut für Hämatologie

Nr. des Vertrages: 089-72-1 BIAD

Leiter der Forschungsgruppe:

Priv.-Doz. Dr. S. Thierfelder

Allgemeines Thema des Vertrages:

Strahlenbiologische Hämatologie und Immunologie
(Proj. 1-3)

(Proj. 4-7 über Nuklearmedizinische Hämatologie sind unter Kapitel V "Forschungstätigkeit Anwendungen Medizin" aufgeführt. Am Schluß dieses Teils des Berichtes befindet sich eine Aufstellung der Publikationen)

Allgemeine Darstellung der durchgeführten Arbeiten:

With the installation of opposing Cobalt cources in the GSF the research group in Munich intensified their studies on the analysis and treatment of the consequences of radiation exposure. Combined conditioning treatment including cyclophosphamide and antisera against thymocytes found effective in mice are being studied in dogs. A first patient with leukemia was treated by antileukemic drugs and total body irradiation of around 850 r followed by bone marrow transplantation from an HL-A- and MLC-identical sibling. Induction of complete remission of the patients' leukemia which had no longer responded to conventional treatment could thus be achieved.

Bone marrow biopsies in locally irradiated patients with genital cancer revealed a defect of the irradiated tissues with a diminution of the marrow's sinusoides together with inconstant inflammatory changes up to 3 years later.

The enhancing conditioning effect of a treatment with ATG which reduces the radiation dose necessary for engraftment by about 150 r could clearly be demonstrated in mice. Because of the promising results in manipulating

secondary disease with anti-T-cell globulin in mice, considerable efforts were concentrated on the production of an anti-human T-cell globulin which no longer cross-reacted with hemopoietic precursor cells in vitro.

Collaborative research in histocompatibility typing was successfully continued on the european level. Our group contributed to the definition of 6 MLC alleles (HLA-DW1-W6) of the genetic locus HLA-D as defined on the 6th International Histocompatibility Workshop Conference 1975 in Aarhus/Denmark. Recently 3 further types were defined by our group: HLA-D type Bo, EI and RE.
Bone marrow transplantation in canine donor-recipient combinations with unilateral incompatibility permitted to dissociate the consequences of host-versus-graft- and graft-versus-host reactions.
The application of quantitative radioautography to the kinetics of bone marrow cell proliferation in preleukemia and overt leukemia permitted new conclusions concerning the mechanism of the amplification of the leukemic blast cell pool. Thus bone marrow transplantation will profit from a better understanding of the changes in the proliferation kinetics of leukemic and pancytopenic patients.

Ergebnisse des Projekts Nr. 1 und 2 (zusammengelegt)

Leiter des Projekts und wissenschaftliche Mitarbeiter:
S. Thierfelder, H. Kolb und H. Rodt

Titel des Projekts:
Partial body irradiation and other non-lethal conditioning treatments of bone marrow recipients.

Combined conditioning treatment with antilymphocytic serum (ALS) and total body irradiation was studied in mice. We could demonstrate in earlier experiments that ALS given before irradiation enhances the engraftment of murine marrow in rats. We observed the same phenomenon after allogeneic transplantation. A 3 days' treatment with o,25 ml ALS decreased the dose of irradiation necessary to induce chimaerism for about 15o R (^{137}Cs, 6o R/min). The study on the effect of ALS and/or irradiation on host-versus-graft reactivity was continued in the lymphnode weight assay. This system measures a local host-versus-graft reaction. It demonstrated residual host-versus-graft reactivity surviving total body irradiation up to 12oo R which was ALS-sensitive and did not occur in donor-recipient combinations, where hybrid resistance was observed.
These studies stimulated our interest in combined conditioning treatment in dogs using ALS, cyclophosphamide and irradiation. The production of a partially purified ALG which can be applied intraveneously in dogs was completed.

Ergebnisse des Projekts Nr. 3

Leiter des Projekts und wissenschaftliche Mitarbeiter:
R. Burkhardt, E. Beil und U. Hahner

Titel des Projekts:
Bone marrow histology in patients treated with radiation, isotopes and radiomimetic agents.

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1. In pursuit of our studies, which have been reported earlier, the late changes of the human bone marrow due to local gamma irradiat have been followed in 25 new patients with genital cancer. The st will be continued.
Preliminary results have been reported on the VII. Akademische Ta gung deutschsprachiger Hochschullehrer in der Gynäkologie und Geb hilfe, Munich, June 18, 1975 and Klinische Wochenschrift (in pres It has been shown that the structural regeneration of the bone ma begins after an interval of average three months from the last irradiation with a total focal dose of at least 3ooo r. The regen ration remains incomplete during the course of the following 3 ye accompanied by a significant diminution of the number of the sinu oidal vessels, and a more or less severe chronic inflammatory reaction with increase of plasmocytes and lymphocytes. In two patients, the late changes could be documented 23 resp. 25 years the radiation. One showed a nearly normal, the other a clearly diminished marrow population. The changes of the cancellous bone, however, clearly demonstrable in the early course of the radiatic damage, have disappeared in the late cases, with the exception of four, showing increased volumina of the osteoid substance, and one with generalized osteosclerosis 6 years from a dose of 55oo r From these observations it is evident that there is only a partia regeneration or recolonization of the bone marrow after the appli cation of a local tumor dose, even when the whole of the haemato- poietic system has remained intact. That means that there must be a defect of the local tissue which is transferable from one cell generation to the other. The only visible manifestation of such a defect is the diminution of the marrow sinusoids in combination with inconstant inflammatory changes. Further investigations are planned in order to elucidate the nature of the defect, and to

compare the atrophic changes of the bone marrow from irradiation with the idiopathic ones. Another subject is to find out, whether the wide range of individual reaction against the radiation should be related with the therapeutic effect and the stage of the disease.

2. The long term study of the bone marrow changes in polycythaemia vera, treated with 32^{P}, has been continued. 65 new cases could be added to a total of 233 cases. The histological follow up includes 50 cases with two or more histobioptic investigations. The data, concerning the morphometric osseous changes, the cellularity of the marrow, the number and changes of blood vessels, and changes of the stromal tissues have been stored for computerized evaluation.

3. In a new series of investigations, the long term effect of cytostatic chemotherapy on the bone marrow tissues is to be observed in cases with neoplastic marrow distortion. Up to now 57 cases are under control.

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Decrease and Altered Distribution of Human T-Antigen on Chronic Lymphatic Leukemia Cells of T-Type Referring to Clonal Origin.
Blood

Associato della Commissione: Comitato Nazionale per l'Energia Nucleare.

No. del Contratto: 108-72-1 BIOI.

Capo del Gruppo di Ricerca: Prof. G. Doria.

Tema generale del Contratto: Protezione e riparazione del sistema immunitario dal danno delle radiazioni.

Collaboratori scientifici: Dr. G. Agarossi e Dr. G. Gorini.

Protection of the individual from invasion and pathogenic action of microorganisms relies mainly on the efficiency of the immune system. The immunologic surveillance results from antigen recognition and from humoral and cellular responses. These biological processes require the partecipation of cell populations, such as macrophages, B, and T lymphocytes, each endowed with characteristic properties. Size and functions of these cell populations vary with age and are altered by radiations and other environmental agents. Such variations affect the protective functions of the immune system.

Recovery of antibody affinity after a sublethal dose of X-rays. In several animal species spontaneous recovery of the antibody response is complete within 2 months from exposure to a large sublethal dose of X-rays. As we previously reported, recovery of the secondary response was studied in mice given 450 R, primed either immediately or 30 days after irradiation and challenged with the same antigen at different times. When the irradiated immune system was allowed to recover for 30 days before priming the secondary response to antigen given after 10, 20, 30, or 60 days resulted in normal levels of antibody concentration but in higher affinity than that in unirradiated controls. When the animals were primed immediately after irradiation antibody affinity was the same as in normal controls while the antibody concentration was subnormal.

A similar study was undertaken on the recovery of antibody affinity

during the primary response elicited at different times from irradiation. Purpose of this investigation was to analyze a simpler condition than the secondary response, which can be more directly correlated with specified cell populations that could have recovered from radiation damage at the time of immunization. C3H mice that received a total body X-ray dose of 450 R were immunized I.P. with 0.1 mg of alum precipitated DNP-KLH and $1x10^9$ B. pertussis, immediately (group A), 1 week (group B), 2 weeks (group C), 3 weeks (group D), or 4 weeks (group E) after irradiation. A group of unirradiated mice was immunized as above and served as control. Eight mice of each group were sacrificed at weekly intervals for 4 weeks and at each time sera were pooled for determination of the concentration and affinity of antibodies specific for DNP. Both measurements were performed by equilibrium dialysis technique whereby immunoglobulins prepared by serum precipitation with 40% ammonium sulfate were reacted against several concentrations of tritium-labelled DNP-lysine. From binding data at equilibrium the antibody concentration was estimated as moles of total combining sites per liter of serum and the antibody affinity as the reciprocal of the DNP-lysine concentration at which 50% of the combining sites are saturated. The rate of antibody production was found lower in groups A, B, and C whereas it approached the normal value in groups D and E, suggesting that recovery of the immune system was almost complete at 3 weeks from exposure to 450 R. Antibody affinity was slightly below normal values in group A, but it increased at faster rates than normal in groups B, C, and E. The value attained in group E at 4 weeks after immunization was 5 fold greater than that in unirradiated controls. Thus, starting from 1 week after exposure to 450 R the immune system can produce antibodies with affinity appreciably higher than that of antibodies raised in normal animals.

The results of this study on the primary response to DNP extend the previous study on the secondary response to the same antigen, demonstrating that after a sublethal dose of X-rays spontaneous repopulation of the immune system favours the capacity to produce high affinity antibodies. Among the cellular events that may account for this phenomenon, changes in the T and B

cell populations might be relevant, as it is known that the B cell population can produce antibodies of higher or lower affinity upon interaction with helper or suppressor T cells, respectively. Thus, a kinetic study of T and B lymphocytes in the spleen of sublethally irradiated mice was undertaken. C3H mice were sacrificed at weekly intervals after a total body X-ray dose of 450 R and their spleen cells cultured *in vitro* with mitogens specific for B (LPS) and T (ConA and PHA) cells. Responsiveness to each mitogen was measured by cell incorporation of tritium-labelled thymidine. The response of normal spleen cells to ConA was greater than the responses to PHA and LPS which were approximately equal. Preliminary results showed that the response of spleen cells harvested from mice immediately or 1 week after irradiation was negligeable for all mitogens. Two weeks after irradiation, all responses were subnormal, but the response to LPS was greater than those to ConA and PHA. These data indicate that recovery of B cells is faster than that of T cells with a consequent shift of the lymphocyte population in favour of B cells. A relative lack of suppressor T cells may account for the increase of antibody affinity observed when antigen was injected at 2 weeks or at subsequent times after irradiation.

Biological effects of E. coli lipopolysaccharide (LPS) *in vivo*. LPS is the principal component of endotoxins which are produced by gram-negative bacteria and released into the surrounding medium if the microorganisms undergo disintegration or lysis. Injection of endotoxin produces several biological effects, one of which is the adjuvant effect demonstrated in normal as well as in sublethally irradiated animals. If injected after irradiation endotoxin is, indeed, a potent restorative agent for the immune system. LPS from E. coli has a mitogenic effect on B lymphocytes *in vitro*. But there is evidence that LPS *in vitro*, besides stimulating directly B lymphocytes, may also stimulate helper T cells. LPS *in vivo* seems to influence T lymphocytes since it enhances allograft rejections, graft-versus-host reactions, and delayed hypersensitivity. Whether LPS *in vivo* has definite effects on size and functions of a T cell population was investigated in the mouse thymus. A single I.P. injection of

LPS (0.1 - 100 µg) into BDF1 mice produced the following effects on the thymus: reduction of the organ weight and depletion of the cortex; decrease of theta-positive cells; increased killer and helper activities of thymocytes as shown by transfer onto irradiated recipients; increased responsiveness of thymocytes to PHA and ConA *in vitro*. The enhanced sensitivity to these mitogens was different for PHA and ConA, and dependent on the dose of LPS injected. The kind of dose dependency observed suggests that LPS *in vivo* modifies the thymocyte population by progressively eliminating immature T1 cells and selecting more mature T2 cells. The data on thymocyte responsiveness to mitogens support the T cell dependency of the adjuvant properties of LPS because killer and helper cells belong to the T2 whereas suppressor cells to the T1 cell subpopulation. These effects on the thymocyte population seem to be mediated by corticosteroids because they were prevented by adrenalectomy, a finding in agreement with the notion that the injection of cortisone induces similar cell changes in the thymus.

Age-dependent variations of antibody avidity. Antibody avidity, a function of affinity, determines the stability of antigen-antibody complexes. It is the property that conditions the antibody capacity of neutralizing viruses and toxins. Antibody avidity is influenced by changes in size and functions of B and T cell populations. Since these populations vary with the age, a study was carried out in C3H mice to detect changes of antibody avidity as a function of age. Spleen cells from donors of different ages (10 - 720 days) were transferred and stimulated with TNP-HRBC in lethally irradiated syngenic recipients. The immune response of the donor cells against TNP was estimated from the number of direct PFC per recipient spleen by the Jerne technique with TNP-SRBC. Avidity of antibodies secreted by PFC was evaluated from the amount of added TNP-BSA that inhibited 50% of the PFC anti-TNP. Under these experimental conditions age-dependent variations of antibody response and avidity can be attributed to changes in the spleen cells rather than to changes in their original environment. Regardless of the donor age, a positive linear relationship, on log scales, was found between number of PFC and

number of transferred cells and between antibody avidity and number of PFC. This indicates that avidity depends on the magnitude of the antibody response. But, each linear regression has different coefficients for different ages, thus making it difficult to compare avidities of different ages. However, from these data avidity appeared to vary parabolically with age. After appropriate correction of the number of PFC to make it independent from age, avidity values were fitted by a multiple curvilinear regression in which the independent variables playing a significant role, as estimated by stepwise analysis of the regression, were the number of PFC in its linear term and the age in its linear and quadratic terms. Comparisons of the standard coefficients from this regression showed that the observed variations of avidity can be attributed in part (80%) to the number of PFC and in part (20%) to the age. For any given number of PFC, the curvilinear regression describes the variations of avidity due only to age: the value of antibody avidity at 10 days increased 15 fold to reach a maximum at 110 days and then declined 5 fold at the age of 720 days.

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Biological effects of escherichia coli lipopolysaccharide (LPS) *in vivo*. I. Selection in the mouse thymus of killer and helper cells. Submitted.

ADORINI L., RUCO L., UCCINI S., SORAVITO DE FRANCESCHI G., BARONI C.D., DORIA G.
Biological effects of escherichia coli lipopolysaccharide (LPS) *in vivo*. II. Selection in the mouse thymus of PHA and ConA responsive cells. Submitted.

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Effect of interaction between hapten-specific cells preselected for different receptor affinities and carrier-primed cells on antibody avidity.
In: Fifth Intern. Conf. on Lymphatic Tissue and Germinal Centers in Immune Reactions., Plenum Publ. Co., in press.

Contractant de la Commission :
UNIVERSITE LIBRE DE BRUXELLES

N° du contrat :
093-72-1 BIOB

Chef des groupes de recherche :
Jacques E. DUMONT

Thème général du contrat :

Definition of the methodology for the study of the effects of radiation on human tissues (blood, cells, etc.) and application of this methodology.

The general aim of the project is the study of physiological and biochemical mechanisms, the alteration of which causes the short and long term effects of radiation and to develop the methodologies necessary to investigate these mechanisms..

The problems studied are the regulation of erythropoiesis, polymorphonuclear phagocytosis and the development of a mathematical model of thyroid follicular cell irradiation by radioisotopes of iodine.

A. ERYTHROPOIETIN

Irradiation affects erythropoiesis and the catabolism of erythropoietin. The main results obtained in 1975 were :

1) Further proof that the decrease in erythropoietin catabolism in irradiated animals is not due to bone marrow aplasia has been provided.
2) The mechanism of erythropoietin catabolism by kidney parenchyma has been investigated.
3) Arguments in favor of the hypothesis that androgens act on the human kidney parenchyma to stimulate erythropoietin have been provided.

B. POLYMORPHONUCLEAR PHAGOCYTOSIS

Irradiation causes both a leukopenia and a decreased bactericidal activity of the leucocytes. The main results obtained in 1975 were :

1) The development of new protocol for the study of irradiation effects in man.
2) New evidence in favor of the hypothesis that the biochemical concomitants of phagocytosis are due to the formation of a signal at the level of the plasma membrane have been provided.

C. MODEL OF THYROID FOLLICULAR CELL IRRADIATION

This program is carried out in another contract.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques
J.P. NAETS, M. WITTEK, C. DELCROIX

Titre du projet :

Effects of irradiation on the regulation of erythropoiesis.

Two effects of irradiation on hematopoiesis are known : a general depression of hematopoiesis and a decrease of the catabolic rate of erythropoietin (Proceed. Soc. Ecptl. Biol. Med., 10, 40, 1959). Two aspects of these problems are studied : the control of erythropoiesis and its therapeutic implications and the metabolism of erythropoietin.

A. ERYTHROPOIETIN METABOLISM

We have shown that irradiation increases the half life of exogenous and endogenous erythropoietin, i.e. that it slows down its catabolism. Our first hypothesis to explain this effects was that the target tissue, the bone marrow could catabolize its regulatory hormone, as the liver inactivates glucagon ; the decreased catabolism of erythropoietin after irradiation would then be a consequence of medullary aplasia. The definition of the dose response relationship neither proved nor disproved this hypothesis. In a first series of experimentations it was shown that aplasia induced by an alkylating agent mustine (nitrogen mustard) was not accompanied by a decreased rate of erythropoietin catabolism. Cyclophosphamide (Endoxan) induces a more intense depression with less lethality. The kinetics of the restoration of erythropoiesis is parallel after X ray and cyclophosphamide treatment which allows a valid comparison. Under such rigorous conditions there is no decrease in the catabolic rate of erythropoietin. The action of X rays on the catabolism of erythropoietin therefore indenpendent of their action on the bone marrow.

We have shown in vivo that kidney parenchyma is involved the catabolism of erythropoietin (J. Lab. Clin. Med., 84, 99, 1974). To investigate this metabolism, dog kidneys were perfused with autologous erythropoietin rich plasma in a closed circuit system (Nizet). Contrary to previous preliminary results after 2 hours of perfusion the erythropoietin level was not decreased. These experiments therefore fail to reproduce the in vivo kidney action. However, it is possible that the kidney may alter erythropoietin in such a way (e.g. by desyalidation) that the hormone would retain its activity but become more susceptible to degradation in other organs. This hypothesis will be checked by injecting dogs and rats with kidney perfusate.

Plasma from hypoxic rats seems to induce erythropoietin formation in normal plasma. For example, a plasma containing 7U/ml of erythropoietin when diluted 10 fold and incubated with normal plasma (0U/ml) contains 2-3U/ml. This suggests that 1.6U of erythropoietin activity has been generated. It has already been postulated that the kidney could contain a factor capable of generating erythropoietin from normal plasma. We shall try to demonstrate the existence of this factor in the kidney and in the plasma of severely hypoxic rats.

B. CONTROL OF ERYTHROPOIESIS

Erythropoiesis, as evaluated by the amount of transfused blood which is necessary to keep up their hematocrit is much reduced in patients with renal failure. We have shown that androgens have no effect on erythropoietin plasma level or blood requirements in anephric patients but decrease them in patients with renal failure but with some parenchyma left. These data suggest that the action of androgens in enhancing erythropoiesis bears on kidney parenchyma.

PUBLICATIONS

1) J.P. NAETS.
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2) J.P. NAETS.
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3) A. VERHEST, J. VANSCHOONBROECK, M. WITTEK, J.P. NAETS, R. DENOLIN-REUBENS.
The specificity of the 5q-chromosome in a distinct type of refractory anemia.
J. Natl. Cancer Inst. (in press).

4) J.P. NAETS.
Compared effects of irradiation and nitrogen mustard induced erythroid aplasia on the catabolism of exogenous erythropoietin.
Submitted to Brit. J. Haematology.

Résultats du projet n° 2

Chef du projet et collaborateurs scientifiques :
E. SCHELL-FREDERICK, R. PARIDAENS,
L. DUBOIS, J.E. DUMONT.

Titre du projet :

Polymorphonuclear phagocytosis.

Phagocytosis by polymorphonuclear leucocytes represents th major defense of the organism against bacterial invasion. Our work concerns the biochemical mechanisms of uptake and killing of bacteria by these cells, with particular emphasis on the con trol elements involved and on genetic and acquired defects in phagocytic function.

A. IRRADIATION

We have previously drawn attention to the fact that leuco- cytes from animals irradiated in vivo demonstrate decreased pha gocytic function prior to the development of leucopenia (Nature 210, 158, 1966) and proposed an evaluation of phagocytic functi as a potentially more sensitive test for low dose radiation dam ge (Euratom report 1974).

We have initiated an experimental protocol in human subjec undergoing irradiation for malignant tumors. The patients have been divided into groups according to the body area irradiated and whether or not active haematopoietic sites are exposed. Pol morphonuclear leucocytes are isolated from subjects prior to, during and after the period of X-ray exposure. The rate of upt ke of particles and the overall activity of the myeloperoxidase enzyme system are evaluated.

Although it is premature to evaluate the results, this pro tocol appears to be superior to that in irradiated guinea pigs as each patient serves as his own control. In order to measure the rate of uptake of phagocytic particles, we have used the sensitive and quantitative technique of Stossel (J. Clin. Inves 51, 615, 1972). Thus the evaluation of phagocytic function in irradiated subjects detects possible defects both in engulfment and killing capacity.

B. CELLULAR MECHANISMS OF PHAGOCYTOSIS

The evidence available suggests strongly that the metaboli counterparts of phagocytosis, including the bactericidal mecha- nisms, are the result of a signal or signals generated by the contact between the phagocytic particle and the cell surface. Studies using cytochalasin B (CB), which inhibits a wide variet of cellular movements, have provided new evidence for such a si gnal. In guinea pig peritoneal leucocytes CB 5µg/ml completely

abolished ingestion of particles. However, it inhibited only partially the stimulation of O_2 consumption demonstrating that during phagocytosis this stimulation is independent of particle entry. CB also prolonged the response time following particle addition, suggesting a partial interference with particle-cell surface contact.

We have continued our work on the possible role of Ca^{++} as a signal in the phagocytic process. Work carried out in 1974 (Euratom report 1974) showed that induction of calcium movement in the absence of phagocytic particles, using the ionophore A23187, mimicked the stimulated oxidative activities characteristic of phagocytosis (FEBS Letters, 48, 37, 1974). Recent results show that phagocytosis is associated with changed calcium flux in leucocytes prelabeled with ^{45}Ca, thus strengthening the possibility that Ca^{++} may be a mediator of the phagocytic process.

PUBLICATIONS

1. E. SCHELL-FREDERICK, R. PARIDAENS, L. DUBOIS.
The immediate consequences of particle-cell contact in PMN leucocyte phagocytosis.
International Congress of the Reticuloendothelial Society, September 1975 (abstract).

2. R. PARIDAENS, L. DUBOIS, E. SCHELL-FREDERICK.
Effects of cytochalasin A, B, D and E on phagocytosis by polymorphonuclear leucocytes.
Submitted to J. Reticuloendoth. Society.

Contracting Research Institute:	Radiobiological Institute TNO RIJSWIJK The Netherlands
Number of contract:	149-75-1-BION
Head of the research team:	Dr. I. Betel
General subject of the contract:	Early detection of mixed lymphocyte reactivity in the rhesus monkey.

General description of the project:

1. To investigate whether increases in protein or RNA synthesis or other metabolic events can be measured during the first 48 hours of a mixed lymphocyte reaction.

2. To investigate whether eventually occurring early changes can substitute for the "classical" thymidine incorporation as a measure for reactivity.

Results of project No. 149-75-1

Head of the team and co-workers: Dr. I. Betel, Dr. K.J. van den Berg, Mrs. J. Martijnse.

Title of the project: Early detection of mixed lymphocyte reactivity.

1. Early uridine incorporation into RNA. Synthesis of ribonucleic acid (RNA) precedes DNA synthesis and mitosis in lymphocyte blast transformation. MLC's were set up with 10^6 cells of both parties. Tritiated uridine (0.5 - 2 uCi) was added during the last 6 hours of culture. Increases in uridine incorporation were between 18 and 40 % after 24 hours of incubation. In ten experiments one false negative was encountered. No relation between the increase in uridine incorporation and thymidine incorporation was found. If in a larger series these results can be confirmed this seem to be an attractive method to rapidly detect MLC reactivity.

2. Early protein synthesis. Also protein synthesis precedes DNA synthesis and proliferation in MLC.

a) Synthesis of excreted protein.
It has been reported by Parsa and Kountz (Nature 250, (1974), 675) that a major portion of the increase of radioactivity incorporated in protein during the early phase of a MLC reaction is associated with proteins excreted in the medium. In an extensive series of experiments with Rhesus lymphocytes, we have been unable to reproduce their results.

b) Stimulation of intracellular protein synthesis.
A relatively simple semimicroassay has been described by Adkinson et al. (J. Immunol. 112, (1974), 1426). In this assay 10^6 cells of both donors are incubated in leucine and isoleucine free medium. The cultures are labelled by addition of ^{3}H-leucine during the last 2 - 4 h of culture. Increases in leucine incorporation at 24 hours of culture were not consistently observed in our system and the increases found were rather small (10 - 20 %). Background values were rather high and variable from donor to donor.

From the results ontained so far it seems that uridine incorporation into RNA is the more attractive candidate for the early detection of MLC reactivity.

LANGZEITWIRKUNGEN UND TOXIKOLOGIE DER RADIOAKTIVEN ELEMENTE

LONG-TERM EFFECTS AND TOXICOLOGY OF RADIOACTIVE ELEMENTS

EFFETS A LONG TERME ET TOXICOLOGIE DES ELEMENTS RADIOACTIFS

Weitere Forschungsarbeiten zu diesem Thema werden auch in folgenden Jahresberichten beschrieben:

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

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

096-BIOB Univ. Louvain (Goffeau)

Biology Group Ispra

Contractant de la Commission :
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE - MOL

N° du contrat : 095-72-1-BIOB

Chef des groupes de recherche : Jean R. MAISIN

Thème général du contrat : PROGRAMME DE RECHERCHES AYANT POUR OBJET LES EFFETS A COURT ET A LONG TERME DES RAYONNEMENTS

The research performed on this contract have been devoted to the following problems :

SHORT TERM EFFECTS

1. The development of biochemical indicators of radiation damage.
2. Effects of X-irradiation and radiomimetic substances on the synthesis of ribosomal and messenger RNA's and on the structure and formation of polyribosomes.

LONG TERM EFFECTS

1. Influence of chemical radioprotectors on the long term effects of ionizing radiation.
2. Studies on biochemical parameters in different organs.

GENETIC EFFECTS

1. Study of the chromosome rearrangements induced in male mice by ionizing radiations.
2. Study of the chromosome rearrangements induced in female germ cells by ionizing radiations.
3. Study of radioinduced chromosome aberrations by banding pattern techniques.

Résultats du projet n° 1
Chef du projet et collaborateurs scientifiques : G. GERBER
Titre du projet : BIOCHEMICAL INDICATORS OF RADIATION DAMAGE

The studies on biochemical indicators of radiation damage were continued measuring about 30 potentially useful compounds in the urine of partially body irradiated rats. The rats were exposed either to the thorax or the abdomen with doses from 200 to 2000 RX rays. The data confirmed the earlier findings in whole body exposed animals showing that in rats excretion of creatine, deoxycytidine and taurine reacts most readily. Abdominal irradiation appeared to be more effective in this respect than thorax irradiation. After the experiments in urine had been terminated techniques for potential indicators in blood are now assembled in order to edit a manual similar to that for urine. In particular, a gas chromatographic method to determine pseudouridine was developed which speeds up largely its determination.

Other studies dealt with various early effects of irradiation. Absorption of glucose and sucrose by intestine from supralethally irradiated rats was studied using an *in vivo* preparation. An activation of glucose absorption one day after exposure is followed by a pronounced fall in glucose and sucrose absorption. Experiments under different conditions of loading indicate that at 20 hours, active transport of glucose is already impaired although the maximal velocity is increased. After three days maximal velocity and active transport decrease markedly. The defect in sucrose absorption is paralelled by a decrease in saccharase activity. Renal function and distribution of ^{51}Cr-EDTA in intra/extra vascular space was studied in rats suffering from the gastro-intestinal syndrome after supralethal doses of X-irradiation. Urine excretion and glomerular filtration were found to decrease until 50 hr p.i. Urine excretion and, in a less degree, glomerular filtration rate increase then to a peak at 67 hrs before falling off to zero values before death.

The extravascular space was found to be expanded in several organs from 60 hrs on (kidney, liver, stomach, intestine). Only in kidney where weight follows changes in extravascular space, a return to normal values is seen before death. An expansion in extravascular space due to a reduced re-extraction into intravascular space and diminished excretion constant can also be discerned beginning early after exposure on the basis of compartmental analysis of the blood activity-time curves. It is postulated that the changes observed reflect a state of shock developing slowly after irradiation and entering its irreversible stage 60 to 65 hrs after exposure. Relative blood flow in different organs of the supralethality (3 kR) whole body X-irradiated rat was studied using labeled 15 μ microspheres. Immediately after irradiation blood flowing to many parenchymal organs ensues. A second maximum occurs at 45 to 50 hrs and a third one at 60 hrs. In most organs, except in brain and liver, relative blood flow diminishes before death. The genesis of these changes as signs of a slowly developing shock is considered.

The study of late effects in brain after 2 K rad was terminated (α-aminoisobutyrate). In these experiments a temporary depression in β-glucuronidase and cathepsin activity followed by an activation at one month was seen. Somewhat later, acid phosphatase increases. During the intermediate period, DNA and serotonin content and AIB uptake by brain increase whereas AIB uptake by heart and muscle decrease. A series of experiments exposing the animals to 3, 4 and 6 K rad was started early in 1975 and up to now yielded similar but more marked changes.

The investigations on late effects in lung were also continued. The right hemithorax of rats was exposed to 3 kR of X-rays, the animals were sacrificed at different times after exposure up to 9 months, and various biochemical parameters were determined. After a slight early decrease, collagen increased during the fibrotic stage. An increase during fibrosis was also seen for DNA, β-glucuronidase, cathepsin, histamine, serotonin and lipid peroxides. Fibrinolytic activity was found depressed at most time points studied. Another series which has not yet been evaluated has been carried out also after 1 kR. Moreover studies are under way to follow the interaction of radiation with SO_2 exposure in the development of lung fibrosis.

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Becciolini, A., Gerber, G.B. Kinetics of glucose and sucrose absorption by an intestinal *in vivo* preparation. Experientia in press.

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Gerber, G.B., Deroo, J. Absorption of radioactive lead (^{210}Pb) by different parts of the intestine in young and adult rats. Environ. Physiol. Biochem. 5, 314-318. 1975

Résultats du projet n° 2

Chef du projet et collaborateurs scientifiques : R. GOUTIER, W. BAEYENS

Titre du projet : EFFECTS OF X-IRRADIATION AND RADIOMIMETIC SUBSTANCES ON THE SYNTHESIS OF RIBOSOMAL AND MESSENGER RNA's AND ON THE STRUCTURE AND FORMATION OF POLYRIBOSOMES.

We continued the study of the influence of *in vivo* irradiation upon transport of labelled ribonucleoproteins from nuclei of rat liver in an *in vitro* incubation system. First, we examined two parameters of this system which allow extrapolation to the *in vivo* situation. The dependence of nucleocytoplasmic transport upon the incubation temperature and upon energy supply (Fig. 1) indicate that this experimental approach reflects closely the *in vivo* situation.

Several control experiments are performed to exclude unspecific transport due to nuclear lysis or to contamination with labelled perinuclear cytoplasmic components:

a. Morphologic examination of all nuclear preparations by phase contrast microscopy (qualitatively and semi-quantitatively).
b. Detection of DNA solubilisation during incubation after *in vivo* labelling of DNA with ^{3}H-thymidine. This control, performed with regenerating liver, revealed that less than 2 % of nuclear DNA is solubilized during the *in vitro* incubation of nuclei.
c. Colorimetric quantitation of nuclear DNA before and after *in vitro* incubation to check DNA release in normal liver. This control allows correction for unspecific transport due to nuclear lysis.
d. Incubations of a complete system at 0° C to correct for unspecific contamination with labelled perinuclear cytoplasmic components.

After checking the system by these controls which are regularly repeated, we looked after a dose-response relationship in this system. As pointed out in previous work, we have to discriminate between quickly labelled m-RNA and

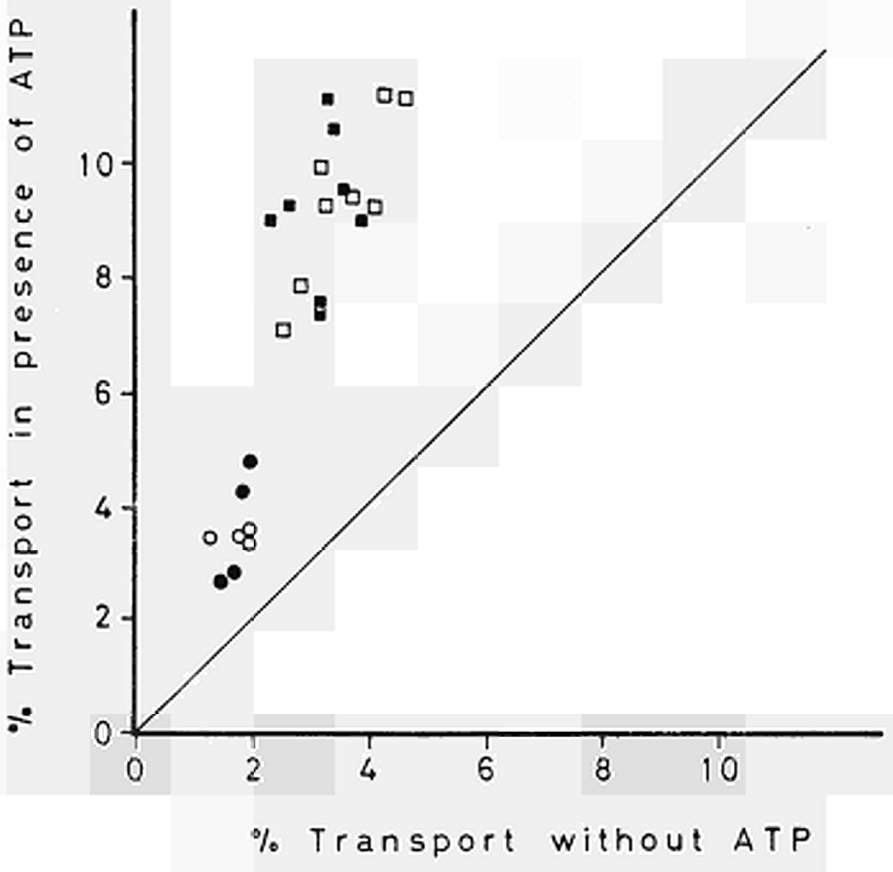
more slowly labelled r-RNA (Figs 2 + 3). The time after irradiation must also be taken in consideration. The first experiments in this series reveal a positive correlation between radiation dose (500 to 2000 R) and enhanced transport of r-RNA 24 hrs after irradiation. On the contrary, shortly after irradiation (3 hrs) we could not demonstrate a dose-dependence for the transport of quickly labelled m-RNA. This series of experiments must be completed with higher radiation doses and repeated at different intervals after irradiation.

In all previous experiments, we used a cytosol fraction derived from normal unirradiated animals. This allows us to detect the specific influence of whole-body irradiation upon transport at the level of the nuclear pore complex of the nuclear membrane. We do not exclude a priori a radiation-induced alteration of the cytoplasmic factors which are very important regulators of nucleocytoplasmic transport of ribonucleoproteins. Shortly after irradiation (3 hrs) we couldn't demonstrate a different stimulation of transport with cytosol derived from irradiated or control animals. The radiation-induced inhibition of transport which is manifest at this time period is clearly determined by nuclear modifications in response to irradiation. This series of experiments must also be completed with the study of the effect at later times after irradiation. The active cytosol fraction must be further purified, DEAE-sephadex chromatography already doubling the transport stimulating activity.

Our previous work revealed a different response of regenerating and of normal liver after irradiation. Differences in molecular control mechanisms of the nuclear activity and in sensitivity to hormonal stimuli after irradiation are probably at the base of this phenomenon. One of the nuclear control mechanisms determines the specific transport of nuclear material out of the nucleus. This selective nuclear restriction is disturbed in some tumors, e.g. hepatomas. This same control mechanism is also radiation-sensitive. For that reason, we started the study of a transplantable hepatoma in collaboration with Dr M. Lemaire (Radiotherapy Unit of the University of Liège).

Finally, the presence in blood of factors influencing the nucleocytoplasmic transport has been demonstrated. We will study their role and relative importance in function of the time after irradiation.

ENERGY-DEPENDENCE OF NUCLEAR TRANSPORT



Labelling time: 30 min.

Irradiation: 2000 R, 24 h. before incubation

○ ● : Nuclei incubated at 0 °C

□ ■ : Nuclei incubated at 30 °C

Fig. 1

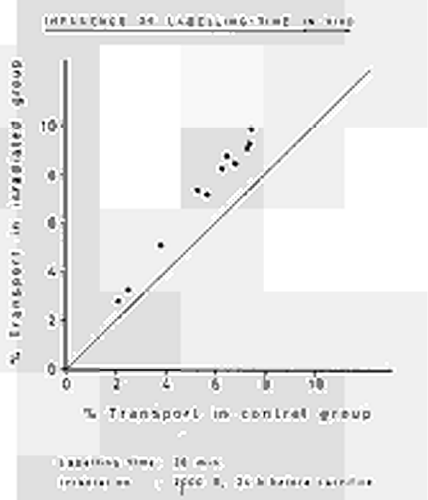

% Transport in irradiated group
% Transport in control group

Fig. 2

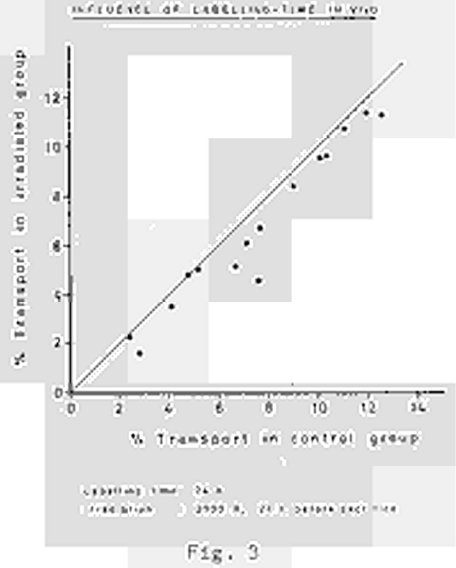

% Transport in irradiated group
% Transport in control group

Fig. 3

Résultats du projet n° 3

Chef du projet et collaborateurs scientifiques : J.R. MAISIN, G. MATTELIN, M. LAMBIET-COLLIER

Titre du projet : INFLUENCE OF CHEMICAL RADIOPROTECTORS ON LONG-TERM SURVIVAL AND CAUSES OF DEATH OF X-IRRADIATED MICE

We have shown previously that :

1. Mixtures of chemical protective drugs increase markedly the short term survival of X-irradiated mice. No information exists, however, in the literature on the protection offered by these mixtures on the colony-forming cells of the bone marrow.
2. BALB/c mice which are treated with a mixture of chemical protectors and given whole-body X-irradiation at supralethal doses (1400 to 2000 R) and which survive the first month after treatment, die within 6 months from radiopneumonia.
3. In BALB/c and C57Bl mice given a single dose of whole-body X-irradiation, sulfhydryl radioprotectors reduce the incidence of thymic lymphomas. This radioprotective effect was, however, less marked for C57Bl than for BALB/c mice.

In this progress report we provide some information on 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 colony-forming cells of the bone marrow of X-irradiated mice on the incidence of radiopneumonia after a single exposure to X-rays and on the incidence of thymic lymphoma after repeated X-ray exposures.

Protection of the colony-forming cells of the bone marrow in mice by mixtures of radioprotectors.

In the first experiment, the LD50/30 days was determined for XVII female and male mice treated with different doses of chemical compounds. In the second experiment, mice were given an initial dose of between 450 and 850 R of X-rays. 24 hrs later

the same mice were injected intravenously with 1 x 10^6 isogenic bone marrow cells and then immediately exposed to a second dose of X-rays of 100, 200, 300 or 400 R. Before the second exposure, half the mice were treated with a mixture of 5 radioprotectors. Ten days after the second exposure mice were killed by cervical dislocation ; the spleens were fixed in Bouin and the number of colony-forming units (CFU) deduced from the colonies formed in 10 spleens per group.

Table I gives the LD50/30 days and the Dose Reduction Factor (DRF) for mice and CFU. The DRF for the CFU of the bone marrow is lower or equal to the DRF obtained for the LD50/30 days survival.

The DRF was calculated from the LD50 and not from the DD. Indeed, the DD does not take into account the shoulder of the slope (The DD in R for the controls, the protected I (P1) and protected II (P2) was 56, 120 and 126 R respectively). Our results demonstrated that the administration of a mixture of radioprotectors provides to CFU of the bone marrow in the optimum conditions a DRF of 2.3 which is significantly lower than the DRF obtained for the LD50/30 days.

Radiopneumonia

BALB/c and C57Bl mice 3 months old were irradiated on the thorax. The mean survival time of non-treated BALB/c and C57Bl mice exposed to doses of between 1200 and 2000 R is very similar. The mean survival time of the treated irradiated BALB/c mice is slightly improved compared to the non-treated irradiated mice. On the contrary the mean survival time of the treated C57Bl mice is markedly improved compared to the treated BALB/c mice. A study of the causes of this discrepancy between the protection offered to C57Bl and BALB/c mice is in progress.

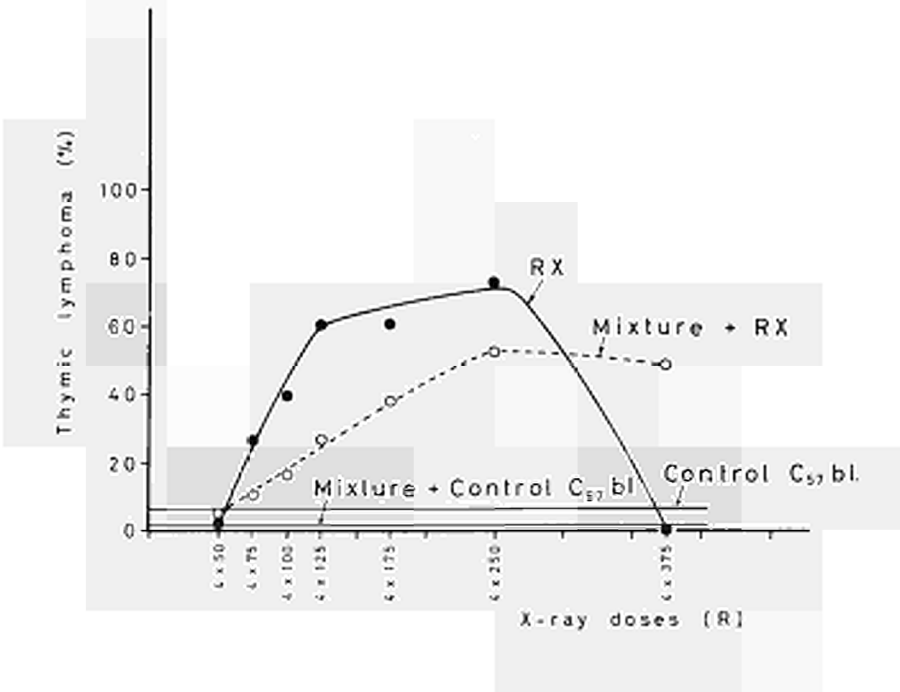
Thymic lymphomas

C57Bl mice 4 weeks old were exposed to increasing doses of X-rays (4 x 50 to 4 x 375 R) given at 8 day intervals. The mortality and the incidence of thymic lymphomas was followed (fig. 1). The results show that in the optimal conditions of thymic lymphoma induction, mixtures of chemical protectors efficiently protect C57Bl mice.

TABLE 1

LD50 and Dose reduction factor for mice and CFU after protection with a micture of radioprotectors.

Protection	Mouse		CFU	
	LD50	DRF	LD50	DRF
Control	685	-	78	-
Protected 1	1600	2.32	178	2.3
Protected 2	1900	2.77	179	2.3



J.R. MAISIN
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J.R. MAISIN, M. LAMBIET-COLLIER, G. MATTELIN
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in : Atomkernenergie (in press).

J.F. DUPLAN, G. MATTELIN, J.R. MAISIN
Protection of the colony-forming cells of the bone marrow in mice by mixtures of radioprotectors.
- Int. J. Radiat. Biol. (in press).

Résultats du projet n° 4

Chef du projet et collaborateurs scientifiques : A. LEONARD, E.D. LEONARD

Titre du projet : STUDY OF THE CHROMOSOME REARRANGEMENTS INDUCED IN MALE MICE BY IONIZING RADIATION

In previous experiments on the persistence of chromosome rearrangements induced in male mice by X-irradiation of pre-meiotic germ cells some observations suggested (Léonard and Deknudt, 1970) that the incidence of spontaneous chromosome rearrangements increased with age in control male mice. Some complementary observations have been performed in order to detect in the male mice a possible relation between the ageing process and an increased number of numerical or structural chromosome aberrations in germ cells which then could eventually be transmitted to the progeny. Cytological observations performed on dividing spermatocytes have showed that 76.83 % of the cells appear normal, 2.8 % - 5.0 % are polyploid, 1.2 - 5.9 % have autosomal univalents and 9.75 % - 14.6 % show X-Y univalents. No reciprocal translocation was observed in the dividing spermatocyte.1. The absence of evident relation between age and the incidence of anomalies suggests that the differences observed result of technical features.

A. LEONARD
Tests for heritable translocations in male mammals.
Mutation Research 31, 291-298 (1975)

A. LEONARD
Ageing and chromosome aberrations in male mammalian germ cells.
Exp. Gerontol. 10 (in the press, 1975)

A. LEONARD
Effects of low levels of ionizing radiations on male mammalian germ cells.
Int. Congress Series N° 338, Radiology 590-592

A. LEONARD
Estimation of chromosome damage induced by ionizing radiations in human germ cells.
Third European Congress of the IRPA, Amsterdam 13-16 May, 3-10, 1975

Résultats du projet n° 5

Chef du projet et collaborateurs scientifiques : A. LEONARD, G. DECAT, Gh. DEKNUDT

Titre du projet : STUDY OF THE CHROMOSOME REARRANGEMENTS INDUCED IN FEMALE GERM CELLS BY IONIZING RADIATIONS

Experiments on the induction of reciprocal translocations in female mouse germ cells have been completed by the study of the fertility of F1 female offspring from irradiated female mice. C57Bl animals were given 0, 25 R, 50 R 100 R or 200 R of X-irradiation (250 kV, 0.25 mm Cu, 100 R/min). Immediately after exposure animals were mated with male mice from the BALB/c strain for a 200 days period. Male offspring from F1 female showing a reduced fertility will be examined for the presence of reciprocal translocation.

In order to be able to extrapolate the experimental results from animals to man some experiments have also been performed on the radiosensitivity of chromosomes from different species. For that purpose, complementary experiments have been performed on the induction of chromosome aberrations in Sus scrofa, the pig 538 chromosomes, 64 chromosome arms), Ovis aries, the sheep (54 ; 60), Capra hircus, the goat (60 ; 60), and Bos taurus, the cow (60 ; 60). The mean area covered by lymphocyte nuclei was estimated by measuring the nuclear diameter of two hundred lymphocytes. The differences were relatively small between the species varying from 30.01 μ^2 in sheep to 37.43 μ^2 in pig, the relative nuclear volume being 1.00, 1.03, 1.06 and 1.24 for sheep, cow, goat and pig respectively. The data for the incidence of radiation-induced chromosome dicentrics exhibited for cow, but not for the other species, significant inhomogeneity between the experiments. The parameters were determined for the quadratic as well as for the power law. From the results it can be stated that pig differs from the other species significantly with respect to the form of

the dose effect regardless which way of presentation is chosen. For the power law, although the RBE of goat relative to sheep is constant, its estimated value (1.133 $\pm$ 0.035) is significantly greater than 1 ; thus there is, in this case also, strong evidence to exclude the suggestion that the lymphocytes of sheep and goat have the same radiosensitivity. The great heterogeneity between experiments for the cow does not permit to obtain a sufficiently great distinction from other species. Nevertheless, it is evident from the log log and lin lin plot of the data, that the radiosensitivity of the four species is clearly distinct from that of man. Brewen et al. (1973, 1974) have postulated that the genetic sensitivity to X-rays is proportionnel to the number of chromosome arms in the nucleus. This theory is very atractive since, when proved, it would give more assurance to the extrapolation of data from rodents to man. There is no doubt also that other factors beside the number of arms might play a role for the probability of exchanges of chromosome fragments between damaged sites. Amount of DNA in the nucleus and nuclear sizes being the two most obvious ones. The species studied by us also do not exhibit differences in volume or DNA content which could explain the differences in radiosensitivity and type of dose effect curve found.

Résultats du projet n° 6
Chef du projet et collaborateurs scientifiques : A. LEONARD, G. DECAT
Titre du projet : STUDY OF RADIOINDUCED CHROMOSOME ABERRATIONS BY BANDING TECHNIQUES

Using whole-body X-irradiation of male mice or exposure to chemical mutagens such as alkylating agents, Roderick and coworkers were able to induce paracentric inversions in mice (Roderick, 1971 ; Roderick and Hawes, 1970 ; 1973). A high frequency of bridges in the first meiotic anaphase was utilized as a first indicator of an induced inversion but they confirmed the presence of pericentric inversions by genetic methods such as the study of genetic recombination between genes located within or near the inverted segment. In a further study (Davisson and Roderick, 1973) cytological evidence was obtained for two genetically defined paracentric inversions, In(1)1Rk and In(2)5Rk by using banding pattern techniques.

In the present study fluorescent banding patterns obtained for the paracentric inversions In(2)5Rk, In(5)2Rk and In(5)9Rk have been analyzed using a microphotometric system (Léonard and Decat, 1975 ; Decat, 1975).

In(2)5Rk

In(2)5Rk has been recovered in the offspring of a male given an exposure to 900 R of X-irradiation. According to Davisson and Roderick (1973) the break points occur within the two negative bands (2D and 2F)" with a portion of the distal band contributing to the widening of the proximal one or at the outer edges of each band, or just beyond, with a complete substitution of the two bands". Examination of the curves observed shows that the peaks correspond to the positive bands whereas the minima correspond to the negative bands. The most interesting conclusion which can be drawn from comparison of the curves a and b is that D' is much wider than D whereas F' is hardly discerned. This observation confirms that the break points occured in the negative region 2D and 2F.

In(5)2Rk

In(5)2Rk was produced by exposure of a male mouse to 850 R of X-irradiation. Comparison of the curves shows that the inversion involves only the peaks B and C whereas the centromeric region (A) and the distal part of the chromosome (D) remain unchanged.

In(5)9Rk

In(5)9Rk has been obtained from post-meiotic male germ cells of animals given 0.2 mg/kg TEM. The frequency of anaphase bridges was found to be as high as 73.70 $\pm$ 2.0 % whereas it was only 19.20 $\pm$ 1.0 % in the In(5)2Rk involving also the chromosome 5. Comparison of the curves obtained from an homozygote for the In(5)9Rk and from an heterozygote for the In(5)2Rk shows that the minimum observed between the peaks B' and D' is much more important in In(5)9Rk. This observation suggests that the size of the inverted segment could be more important in In(5)9Rk than in In(5)2Rk.

A. LEONARD, G.DECAT
Identification of the mouse chromosomes by microdensitometry.
Can. J. Genet. Cytol. (in the press)

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München
Institut für Biologie
Abteilung für Strahlenbiologie und Biophysik
Abteilung für Allgemeine und Experimentelle Pathologie

Nr. des Vertrages: 090 - 72 - 1 BIAD

Leiter der Forschungsgruppe: Prof.Dr.O.Hug
Prof.Dr.W.Gössner

Allgemeines Thema des Vertrages:

Pathogenesis of somatic radiation damage

Allgemeine Darstellung der durchgeführten Arbeiten:

The research program as determined in the contract has been continued during 1975. Some detailed studies could be completed others were started or extended as intended before. The activities concerned the following items

- the metabolism distribution and dosimetry of Lu-177 and other β-emitting radionuclides of rare earth
- the distribution and dosimetry of inhaled Th-227
- long-term animal experiments concerning the late effects

a) of Ra-224 given over 36 weeks to male and female mice
b) of Th-227 and its daughter product Ra-223 in female mice at mean sceletal dose level of 1000 and 2000 rd, given at once or protracted over 36 weeks
c) of low dose of Th-227 in new-born mice
d) of single injection of Lu-177 in young female mice at various dose levels and with low and high amount of stable carrier

- all animals in these experiments have been followed up with regard to chronic tissue damage and oncogenesis by pathological investigations
- special pathomorphological studies concerning the influence of cyclophosphamide and dibutly cAMP on the pathogenesis of radiation induced osteosarcomas

- the role of virus in the pathogenesis of radiation induced osteosarcomas
- stimulation of cell proliferation in epithelial and mesenchymal tissues by isoproterenol
- histogenesis, classification and nomenclature of radiation induced tumors (treated in two workshops)
- continuation of epidemiological studies concerning the late effects after Ra-224 therapy.
- preparation of the second International Symposium on biological effects of Ra-224 to be held in 1976.

Project 1

Late effects after incorporation of bone-seeking radionuclides

W. Gössner, O. Hug, A. Luz, W. A. Müller, E. H. Schäffer

1. Metabolic and dosimetric studies with short-lived α- and ß-emitting bone-seekers

The distribution studies of ß-emitting bone-seekers were concentrated on Lu-177 (half-life 6.7 days, $ß_{max}$ 495 keV), which since a couple of years is used in a few branches of nuclear medicine. After i.p. injection it was deposited mainly in the skeleton (up to 50% of the injected activity) as shown in the autoradiographic picture (Fig. 1). The addition of higher amounts of stable Lutetium effected a high focal deposition ("hot spots") within the RE-cell-system (red pulp of the spleen, liver bone marrow) similar to the distribution of colloidal Plutonium compounds. Thus with increasing amount of stable carrier a considerable reduction of skeletal retention was observed and simultaneously a rise of the deposition in the liver.

A preliminary dose calculation led to a mean skeletal dose of 220mrad after the injection of 1 µCi per kg body weight. At small amounts of concomitant stable carrier (0.5 mg/kg) the dose in all soft tissue organs reached less than 10 per cent of this value (see also Fig. 2).

Comparative distribution studies were also performed with two other radionuclides of rare earths namely Ce-141 and Sm-153.

Distribution studies and dosimetry after inhalation of Th-227 (collaboration with C.E.A., Fontenay-aux-Roses) have been published. The evaluation of the corresponding long-term experiment in our laboratory as well as in C.E.A. is in progress.

2. Tumor induction

The late effects of Th-227 and its daughter product Ra-223 (α-emitter, half-life 11.2 days) has been compared in female NMRI mice 3-4 weeks of age (100 mice in each dose group) at the level of 1 000 and 2 000 rad

Fig. 1 Autoradiography of a sagittal section of a lumbar vertebra of a female NMRI mouse 48 hours after incorporation of 61.0 µCi Lu-177/kg. Accumulation of the radionuclide on the surface of the cortical and spongious bone. Haemalaun. x 375.

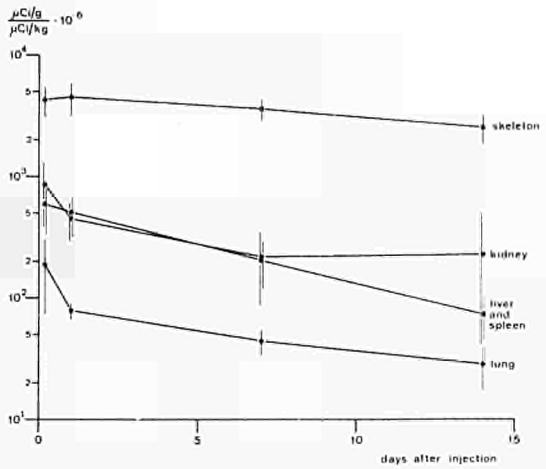


Fig. 2 Concentrations of Lu-177 in various organs per 1 µCi Lu-177/kg body weight as a function of time after incorporation with standard deviations. (Concentration of stable Lu-carrier: 0.5 mg/kg).

mean skeletal dose. The final incidence of osteosarcomas outside of the jaws was about 20% in the Ra-223 experiments and about 50% in the Th-227 experiments. No significant difference between both dose levels was observed in each experiment. The higher osteosarcoma risk after incorporation of Th-227 may be due to its higher accumulation at the bone surfaces and its longer half-life (18.6 days), leading to a more protracted internal irradiation.

Repeated i. p. injections (18 x 0.28 µCi Th-227/kg during 36 weeks, with time intervals of 2 weeks between individual injections, corresponding to a total mean skeletal dose of 1 000 rad) produced osteosarcomas in 80% of the animals. This experiment with female NMRI mice started at 4 weeks of age and resulted in a similar tumor risk as the corresponding experiment with repeated injections of Ra-224 in intervals of 3.5 days.

A long-term experiment was started in which newborn NMRI mice received single i. p. injections of 0.1 or 1.0 µCi Th-227/kg.

The first long-term experiment with single injections of Lu-177 in female NMRI mice, 3-4 weeks of age, is in progress. The animals (50 in each group) received i. p. injections of 5, 10, 20, 40 mCi/kg with stable Lutetium lower than 1 mg/kg (mean skeletal dose 1 000, 2 000, 4 000, 8 000 rad). In addition one group received 30 mCi/kg with 2 mg/kg stable Lutetium, corresponding to a mean skeletal dose of 4 000 rad.

Project 2

Histogenesis, classification and nomenclature of radiation-induced tumors

W. Gössner, A. Luz, E. H. Schäffer

In April 5-6 and November 23-24, 1975 the 7th and 8th workshop of the EULEP-Committee on Pathology Standardization have been organized by our laboratory.

The main topic of the 7th workshop was "Neoplastic and non neoplastic lesions of the nervous system".

The main topic of the 8th workshop was "Neoplastic and non neoplastic lesions of the vascular system and soft tissue".

For further details see EULEP-report.

Project 3

Pathogenesis of early and late effects after internal and external irradiation

V. Erfle, W. Gössner, A. Luz, K.-H. Marquart, E. H. Schäffer, B. Wallner, W. A. Winter

1. Pathogenesis of radiation-induced osteosarcoma

Since early treatment with Cyclophosphamide prolonged the latency time of leukemia in AKR mice (Strauss, Cancer Res. 33, 1724, 1973) we are testing the influence of this substance on the pathogenesis of the radiation-induced osteosarcoma. Female NMRI mice, 3-4 weeks of age, received single i. p. injections of 5 µCi Th-227/kg. 3 to 9 months after incorporatation 50 control animals and 50 experimental animals were treated by monthly injections of 60 mg Cyclophosphamide / kg.

Based on the data given by Cho-Chung in primary and transplantable mammary tumors (Science 183, 87, 1974) we have investigated the influence of Dibutyl-cAMP on the tumor growth of the transplantable NMRI osteosarcoma in a pilot experiment. No significant effect on the growth of this tumor model has been observed.

Stimulation of bone-remodelling during tumor latency time by treatment with Fluoride will be combined with testing of osteosarcoma risk after incorporation of Th-227 in rats in 1976.

Two coworkers visited the Radiobiological Institute of TNO, Rijswijk (Dr. van Bekkum) to study the methods which will be applied in the joint program "Investigation of the immunologic state during tumor latency time", supported by a grant of the Swedish "Stiftelsen Riksbankens Jubileumsfond" (EULEP-project in collaboration with Dr. Nilsson/ Sundbyberg and Dr. van Bekkum/Rijswijk).

Electron microscopic studies of 14 osteosarcomas, induced in NMRI and (C3H x 101)F_1-mice by Th-227, revealed the presence of intracisternal type A virus particles. Additionally, three of these tumors

from (C3H x 101)F_1 mice contained many immature and mature extracellular type C virus particles.

Studies on the presence of oncornavirus in radionuclide-induced osteosarcomas in mice concentrated on:

a) cell culture lines of bone sarcomas in NMRI mice (random bred strain) induced by the incorporation of Ra-224 and Th-227 and
b) transplanted tumors derived from Ra-224-induced bone sarcomas of (C3H x 101)F_1 mice (hybrid of two inbred strains) and NMRI mice.

In all cell cultures tested until now viral particles could be detected. They possess the typical properties of C-type RNA tumorviruses: a density of 1.16 g/ml, a high molecular weight RNA of 70 S and a RNA dependant DNA polymerase (reverse transcriptase) which is complexed to the viral 70 S RNA. By application of the "simultaneous detection test" it was possible to detect tumorvirus RNA associated with a reverse transcriptase in transplanted Ra-224 bone tumors from inbred strain hybrid mice but not in random bred NMRI mice (Fig. 3).

The protracted application of 36 uCi Ra-224/kg over 36 weeks (total mean skeletal 1 000 rad, expected osteosarcoma incidence about 80%) was repeated with male and female (C3H x 101)F_1 mice. The collection and preparation of material performed with the aim to detect oncornaviral parameters in the skeleton during the latency time of this experiment was finished. The analysis of this material is in progress. Since it can not be excluded that microscopic osteosarcoma buds may precede the macroscopic detectable osteosarcomas, we tried to define the latency time in this experiment by the following procedure. During the 9th month of the experiment, i. e. the time period during which the first osteosarcomas appeared, small pieces of bone from 10 experimental animals and 10 control animals were transplanted to 520 syngenic recipients. No development of a transplant tumor was observed in any case.

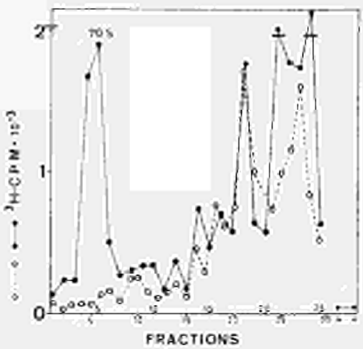


Fig. 3 Velocity sedimentation in 10-30% glycerol of the H3-DNA product from an endogenous reverse transcriptase reaction in virus particles from osteosarcoma cells. The H3-DNA is complexed to the viral 70 S template RNA and sediments as a hybrid complex with the sedimentation behaviour of the 70 S RNA.

●——● sedimentation of the RNA-DNA complex

○- - - - - ○ treatment of the RNA-DNA complex with RNAse before centrifugation

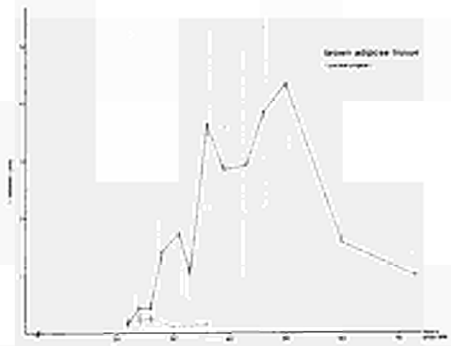


Fig. 4 DNA synthesis in brown adipose tissue after a single injection of 30 mg Isoproterenol ●——● , NaCl-treated controls ○- - -○

2. Stimulation of the cell cycle. Isoproterenol-induced DNA-synthesis in epithelial and mesenchymal tissue

As mentioned in the last report Isoproterenol induced DNA-synthesis in the rat urinary bladder epithelium. In the course of these experiments the effect of repeated injections of Isoproterenol was studied. Over a period of four weeks the animals received 15 mg Isoproterenol i. p. daily. There was no remarkable thickening of the epithelial coating of the bladder, which would indicate an increased turnover of the epithelium and a correspondingly higher cell loss into the lumen. Three to four weeks after the beginning of the experiment only a slight dysplasia of the transitional epithelium was observed. Nine months after a four weeks treatment no tumor could be detected.

Isoproterenol was found to have two effects on the brown adipose tissue in mice. A single injection of 30 mg IPR induces within 8 hours a fall in the fat-content from 50% to 10% of wet weight and a subsequent re-synthesis over a period of 16 hours after which the original fat-content is reached again. 28 hours after IPR injection DNA-synthesis increases to maximal values between 36 and 50 hours (3 to 4%) (Fig. 4).

Project 4

Studies on late effects induced by Ra-224 in children and adults

H.Spiess

The present state of the studies performed in the pediatric policlinic of the university of Munich is given in table 1. As to be seen the registered number of death amongst the observed patient group increased by 42 up to 358, whereby no additional malignant bone tumor occured. However, five osteosarcomas in living persons were newly detected in 1975. The number of carcinomas amounts now to 45. Also the number of benign osteochondromas increased. Eight cataracts may be due to the Ra-224 treatment. Amongst the causes of death a high rate of kidney diseases is remarkable.

Table 1 Reinvestigation of Ra-224 treated patients December 1975

Total number: 1809

not found:241; physicans report without name:654; investigation refused: 16; exactly controlled: 898

Age at beginning of Ra-224 treatment	1-20 years	after 21 years	total number
reply received	218	681	898
report by physicans or questionaire	49	325	374
personally explored or at the hospital observed	107	108	215
deceased	67	291	358

causes of death			
main disease	11	42	53
not in relation to main disease or late effects	11	123	134
unknown	4	31	35
malignant bone tumors,	29 (+3L)	16 (+2L)	45 (+5L)
osteosarcoma,	4	0	4
chondrosarcoma			
other malignant tumors (carcinoma)	3 (+2L)	28 (+12L)	31 (+14)
diseases of the liver (mostly cirrhosis)	1	15	16
diseases of the kidney	3	31	34
panmyelophthisis, anaemia	1	2	3
leukemia	0	3	3
other late effects			
benign bone tumors			
osteochondroma solitary	7	0	7+
multiple	20	0	20+
osteochondroblastoma	1	0	1
fibroma	1	0	1
early broken teeth	19	11	30
delay of growth	50	0	50++
cataract	8	23	31

L = living patients

\+ = of 84 x-ray controls of skeleton

++ = of 99 x-ray controls of skeleton

Project 5

Epidemiological study on late effects after medical application of Ra-224 in ankylosing spondylitis patients

O.Hug, F.Schales

The follow-up of ankylosing spondylitis patients as started in 1971 was continued. Up to now the case reports of 11 German hospitals have been checked, and most of the patients in question could be contacted. Their relevant data have been stored electronically.
About 380 patients have been reinvestigated clinically. The causes of death of about 240 patients could be cleared up.

No case of bone sarcoma was observed so far, but 4 cases of blood diseases, 2 of them being possibly related to Peteosthor application.

Collaboration with other centres and with the EURATOM Thorotrast group was expanded.
Continuous contact with all living patients will be held in the future.

List of publications and reports

Gössner,W.,Hug,O.,Luz,A.,Müller,W.A.: Experimental induction of bone tumors by short-lived bone-seeking radionuclides. Recent Results in Cancer Research, Vol.54, 36-49 (1975).

Luz,A., Gössner,W.: Anatomo-pathological aspects of lesions occurring in long-term experiments after incorporation of internal emitters. Scientific Workshop on 'Problems and Methodology of Experimental Evaluation of Biological Effects of Radionuclides', Brüssel, Belgien, 14.-15.4.1975.

Luz,A.: Problems with regard to the standardized evaluation of long-term experiments in mice. Seminar, National Center for Toxicological Research (NCTR), Jefferson, Arkansas, USA, 24.10.1975.

Luz,A.: Bone tumor induction after incorporation of Ra-224 and Th-227 in mice. Group-Seminar, Argonne National Laboratory, Argonne, Illinois, USA, 31.10. 1975.

Luz,A., Müller,W.A.,Gössner,W., Hug,O.: Estimation of tumor risk at low dose from experimental results after incorporation of the short-lived bone-seeking α-emitters Ra-224 and Th-227 in mice. International Symposium on Biological Effects of Low Level Radiation Pertinent to Protection of Man and his Environment, Chicago, Illinois, USA, 3.-7.11.1975 (in press).

Luz,A.: Experimentelle Daten zur Dosisbeziehung und Pathogenese des Osteosarkoms nach Inkorporation kurzlebiger Alpha-Strahler. Seminar, Kernforschungszentrum Karlsruhe, Institut für Strahlenbiologie,2.12.1975.

Marquart,K.-H.,Erfle,V., Luz,A., Gössner,W.: Über das Vorkommen von Viruspartikeln in strahleninduzierten Osteosarkomen der Maus. Verh. dtsch.Ges. Path. 59, 484 (1975).

Marquart,K.-H.: Early ultrastructural changes in osteocytes from the proximal tibial metaphysis of mice after the incorporation of Ra-224. Rad.Res. (in press).

Müller, W.A., Nenot, J.C., Daburon, M.L., Lafuma, J.: Metabolic and dosimetric studies after inhalation of Th-227 in rats with regard to the risk of lung and bone tumors. Rad. and Environm. Biophys. 11, 309-318 (1975).

Müller, W.A., Humphreys, E.R., Szot, Z., Vanderborght, O.: Progress report of Ebony Group (1974) "Boneseeking Isotopes". EULEP Newsletter 9, 5-24 (1975).

Müller, W.A., Linzner, U., Schäffer, E.H.: Studies on the biological behaviour of Lu-177 in mice. Current Topics of Radiation Research (in press)

Müller, W.A.: Standardization of incorporation measurements in cooperative studies - EULEP-experience. Scientific workshop on "Problems and Methodology of Experimental Evaluation of Biological Effects of Radionuclides", Brüssel, Belgien, 14.-15.4.1975.

Müller, W. A. and Luz, A.: Influence of spatial and temporal dose distribution on bone tumor induction after incorporation of osteotropic α-emitting radionuclides in mice. 3rd European IRPA Congress, Amsterdam, Holland, 13.-16.5.1975.

Müller, W.A., Linzner, U., Schäffer, E.H., Luz, A.: Distribution studies after injection of Y-88, Y-90, Ce-141, and Lu-177 into mice, influence of stable carrier and consequence for biological effects. Int. Conference on Molecular- and Microdistribution of Radioisotopes and Biological Consequences, Jülich, 2.-4.10.1975

Schales, F.: Long-term effects of Ra-224. Scientific workshop on "Problems and Methodology of Experimental Evaluation of Biological Effects of Radionuclides", Brüssel, Belgien, 14.-15.4.1975.

Contractual Partner of the Commission:
Prof.Dr. K.E. Scheer: Director of the Institut für Nuklearmedizin am Deutschen Krebsforschungszentrum, Heidelberg

Contract No.: 063-72-1 PST D

Head of the Research Group: Prof. Dr. K.E. Scheer, Director of the Institut für Nuklearmedizin am Deutschen Krebsforschnungszentrum, Heidelberg,

Assistant Head of the Research Group: Prof. Dr. W.J. Lorenz, Institut für Nuklearmedizin, Deutsches Krebsforschungszentrum, Heidelberg.

Coordinator: Dr. G. van Kaick, Institut für Nuklearmedizin, Deutsches Krebsforschungszentrum, Heidelberg.

The contracted research program is to be perfomed at the Institut für Nuklearmedizin am Deutschen Krebsforschungszentrum, Heidelberg, in collaboration with:

Prof. Dr. H. Muth, Director of the Institut für Biophysik der Universität des Saarlandes (Boris-Rajewski-Institut), Homburg,

Prof. Dr. G. Wagner, Director of the Institut für Dokumentation, Information und Statistik am Deutschen Krebsforschungszentrum, Heidelberg, and

Prof. Dr. A. Kaul, Klinikum Steglitz der Freien Universität Berlin, Nuklearmedizinische Abteilung.

General Topic of the Contract:

Research Project "Thorotrast" - Investigations to Evaluate the Long Term Effects Caused by Artificial Radiation in Man (Thorotrast-Patients).

General description of the performed work:
The goal of the research project was set down after mutual agreement had been achieved in the coordinating committee attended by representatives of the Bundesministerium für Forschung und Technologie, the Deutsches Krebsforschungszentrum and the Institut für Biophysik der Universität des Saarlandes, Homburg.

The research project Thorotrast, supported by the Bundesministerium für Forschung und Technologie and EURATOM includes:

1. Biophysical and clinical examinations of Thorotrast carriers and of patients belonging into a control group.
2. Discovering the fate of Thorotrast carriers and patients of the control group who have already died.
3. Use of radiological, serological and immunological diagnostic methods for the discovery of Thorotrast induced neoplasias.
4. Follow up examinations of patients of the Thorotrast group and the control group and investigate the cause of the death of patients having died since the last examination.
5. Statistical analysis of the obtained results.
6. Determination of the chromosome aberration rate in Thorotrast carriers and non carriers, and the dependence of this to the radiation dose.
7. Experimental examinations to analyse the foreign body irritation and study the results of the radiation eminating from the thorium dioxide agglomerates.

Results of the Project No. 1:

Head of the Project: Prof. Dr. K.E. Scheer (Contractual Partner),
Prof. Dr. W.J. Lorenz (Assistant Head of the Project),
Dr. G. van Kaick (Coordinator)

Scientific Collaborators of the Institut für Nuklearmedizin:

Dr. R. Bader, Dr. D. Lorenz, H. Lührs, J. Kilian,
Dr. W. Knapp, Dr. P. Schmidlin.

Statistical evaluation: Prof. Dr. G. Wagner,
Prof. Dr. H. Immich, Dr. H. Wesch.

Project Title:

a) Search for Thorotrast Patients and for Patients of the Control Group.
b) Clinical and Biophysical Examinations of Thorotrast Carriers and Control Patients.
c) Investigating the Final Fate of Deceased Thorotrast Carriers as well as of Patients in the Control Group.
d) Follow up Studies.

Title a) The search for further Thorotrast carriers was continued in one hospital not visited before. 74 cases were found, and the respective control patients were selected. 22 Thorotrast carriers are still living and were invited; nine of them were examined in 1975. 36 persons have died, 16 within 3 years after injection and 20 later than 3 years after injection. 16 persons were not traceable because they live or have died in the DDR.

Title b) In 1975 we examined 63 out-patients: 20 Thorotrast carriers came for the first time, 16 belonged to the follow-up study and 27 patients to the control group.

The data of all examined patients (1442), both Thorotrast and control group, were coded and given into the computer.

Title c) The elucidation of causes of death was continued. The coded data of all deceased Thorotrast and control patients (2145) were given into the computer (Fig. 1-3).

Title d) Altogether 175 patients of the 805 examined Thorotrast carriers have died since their examination. In the control group there are 57 deaths out of 637 examined persons. In the past year 480 examined Thorotrast patients and 410 control patients were asked by letter for their state of health. From these 480 Thorotrast patients 45 have died. In 23 cases the cause of death was a primary liver tumor (19 histologically confirmed), 10 liver cirrhoses or other serious hepatopathies. The remaining 12 patients died from cardiovascular diseases or accidents.

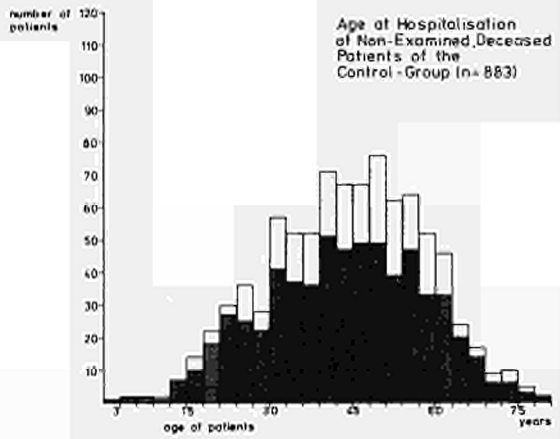


Fig. 1) Distribution of age at time of injection in the group of non-examined deceased Thorotrast patients, (dark = male patients, grey = female patients).

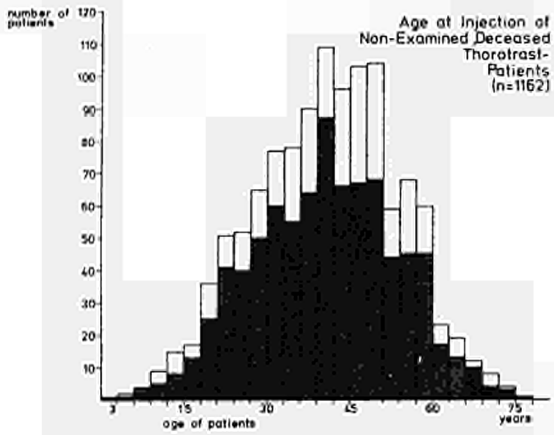


Fig. 2) Distribution of age at the time of hospitalization in the group of non-examined deceased control patients, (dark = male patients, grey = female patients).

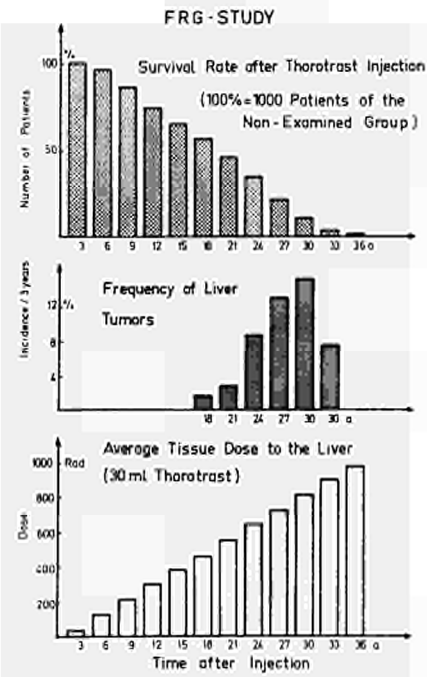


Fig. 3) Survival rate of Thorotrast carriers, incidence of liver tumors, and gradually increase of the total absorbed dose in the liver related to the time after Thorotrast injection.

Publications:

van Kaick, G.:
Late effects of Thorotrast in human population.
Scientific Workshop on Problems and Methodology of experimental evaluation of Biological effects of Radionuclides.
(European Late Effects Project Group (Eulep), Brüssel, 14.-15.4.1975).

van Kaick, G.:
Somatische Spätfolgen schwacher Bestrahlungsdosen.
(Informations- und Schulungsseminar der Kommission der Europäischen Gemeinschaften, Direktion Gesundheitsschutz, Brüssel, 7.-8.10.1975).

van Kaick, G.:
Strahleninduzierte Tumoren.
(Onkologischer Arbeitskreis der Universitätskliniken, Heidelberg, 13.2.1975).

van Kaick, G.:
Knochentumoren bei Thorotrastträgern.
(5. Arbeitssitzung der Internationalen Arbeitgemeinschaft Knochentumoren, Heidelberg, 5.4.1975).

van Kaick, G., Naser. V. u. Lührs, H.:
Spätschäden nach paravasaler Injektion von Thorotrast.
(Deutscher Röntgenkongreß, Berlin, 1.-3.5.1975).

Rogalli, T.:
Untersuchungen zur Klassifizierung und Beurteilung der röntgenologisch erkennbaren Thorotrast-Ablagerungen im retikulohistiozytären System des Menschen.
(Dissertation, to be printed).

Project 2: Working Group Institut für Biophysik der Universität des Saarlandes, 6650 Homburg (Saar)

Head of Project: Prof. Dr. H. Muth

Title a) Clinical and Biophysical Examinations on Thorotrast Patients

Scientific Collaborators: Prof. Dr. H. Muth
Ass. Prof. Dr. W. Kemmer
Prof. Dr. Dr. E. Oberhausen
Dipl.-Phys. A. Steinsträßer

Title b) Chromosome Aberrations caused by Thorotrast

Scientific Collaborators: Ass. Prof. Dr. W. Kemmer
Prof. Dr. H. Muth

Technical Collaborators: U. Welker / G. Metzger

Title c) Radiation and Non Radiation Effects of Thorotrast, in team work with the working groups Prof. Dr. A. Kaul, Berlin, and Heidelberg-Ludwigshafen

Scientific Collaborators: Ass. Prof. Dr. W. Kemmer
Prof. Dr. H. Muth
Dipl.-Phys. A. Steinsträßer

Results of Project 2:

Title a) The clinical and biophysical examinations on Thorotrast patients were continued by the working group at Heidelberg. A field study on Thorotrast patients unable or unwilling to consult the examination institutes at Heidelberg or Homburg will be started in spring 1976.

Title b) and c) To study the important problems of radiation and non radiation effects the experiments announced in the annual report 1974 were realized in 1975. For better knowledge on details of the dose-effect-relationship between radiation dose and chromosome aberrations we started two "in vivo"-experiments with Chinese Hamsters. (1). Three groups of animals were injected with different quantities and qualities of Thorotrast made by the working group at Berlin. The fourth group,

the control group, was injected with physiological solutions only.

First experiment (start Jan. 1975)

Nr	Group I Normal Thorotrast	Group II Th-230 * enriched Thorotrast	Group III Th-230 ** enriched Thorotrast	Control Group 20 % dextrine
1	13.5 µl	13.5 µl	13.5 µl	13.5 µl
2	40.5 µl	40.5 µl	2.7 µl	40.5 µl
3	67.6 µl	67.6 µl	-	67.6 µl
4	135.0 µl	135.0 µl	-	135.0 µl

*) α-energy-emission rate higher by a factor of 5

**) α-energy-emission rate higher by a factor of 50

Duration of experiment: 2.5 ys

The amounts of Thorotrast injected in Hamsters correspond to the amounts used for clinical examinations of patients.

The results of the chromosome aberration analysis obtained in the first experiment will be compared with results obtained in ^{60}Co-γ long time whole body exposure of Hamsters:

Second experiment (start Dec. 1975)

Group	Dose rate (rad/year)
1	80
2	160
3	242
4	327

5 Control group without irradiation

Duration of experiment: 2.5 ys

Independent of the final evaluation of these animal experiments preliminary biophysical and cytogenetic results are in good agreement with results found in Thorotrast patients.

In additional studies we are testing the radiosensitivity of different lymphocyte populations in man.

Publications:

MUTH, Hermann: Radiation Dose and Late Effects from Internally Deposited Radionuclides. Seminar Department of Bioengineering. University of Pennsylvania, Philadelphia, U.S.A., October 10, 1975

KEMMER, Wolfgang: Chromosomenaberrationen als biologisches Dosimeter. Hauptvortrag auf der 9. Jahrestagung des Fachverbandes für Strahlenschutz unter Beteiligung der Vereinigung Deutscher Strahlenschutzärzte. Alpbach/Tirol, 6.-8. Okt. 1975, in print.

Results of Project Nr. 032-67-3 PSTD

Part 3:

Research Group Klinikum Steglitz der Freien Universität Berlin

Head of the Project: Prof. Dr. A. Kaul
Scientific Collaborators: Dr. W. Riedel, T.Dudzus, U.Föll, V.Haase, Prof.Dr. H.J. Stolpmann, U. Walter
Technical Collaborators: J. Franke, B. Müller, B. Rossdorf, S. Schiller, R. Schmidt, G. Witzke

Title of the Project:
Dosimetry, Foreign Body and Radiation Effects of Thorotrast

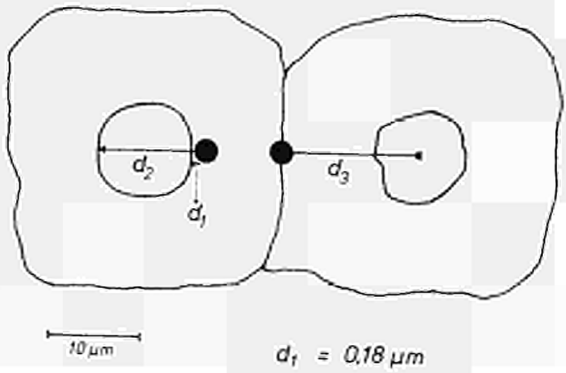
Part 3a:

Tissue Dose from Thorotrast on cellular level

Colloidal $^{232}ThO_2$ is known to be distributed quite inhomogeneously within tissues of thorotrast patients. For reasons of dose calculations with special emphasis to tissue dose on cellular level in man, animal experiments were done, with regard to agglomeration of $^{232}ThO_2$ particles as a function of time after thorotrast injection, and self-absorption of α-particles within thorotrast aggregates of various sizes. 25, 125 and 250 µl of colloidal ThO_2 were injected intraveneously into female mice (average age:8 weeks; average mass: 25g), and each 12 animals from each group were sacrificed between 1 and 100 days after thorotrast administration. Besides γ-ray spectrometry and histo-autoradiografy investigations (whole body and organ measurements of the liver, spleen and bone including marrow) tissue samples were taken for electron microscopy and analyzed with reference to the aggregate size distribution. For dose calculations the aggregates were assumed to be of spherical shape, and classified into 4 groups of 0,045, 0,272, 0,905 and 1,78 µm radius. Using the method of KATO (NIPPON ACTA RADIOLOGICA, Tomus 26 Fasc.12, 25.March 1967), dose rate calculations were made considering self-absorption of α-particles as function of particle size and activity ratios of ^{232}Th and its

α-emitting daughters.

With reference to cellular structures of human liver cells the results of dose calculations can be summarized as follows (see figure 1):



d_1 = 0,18 µm
d_2 = 10 µm
d_3 = 15 µm

Nuclei of liver cells of 10 µm in diameter adjacent to thorium dioxide aggregates of 1,78 µm in radius at a distance of = 0,2 µm will be exposed to α-rays with dose rates between 2 600 (d_1) and 60 (d_2) rads per year. An aggregate of the same size but located at the cellular membrane will irradiate the membrane with a dose rate of about 3 700 rads per year. The dose rate to the nucleus of a liver cell adjacent to the above aggregate at a distance of 15 µm (d_3), is about 30 rads per year. The mean dose rate to cells or cellular structures close to 0,045 to 1,78 µm aggregates (between 0 and 16 µm distance from the aggregates' surface) proved to be 0,2 to 1 600 mrads per year while the average tissue dose rate is as low as 0,2 mrads/year.

Part 3b:

Distribution of ZrO_2 after intravenous injection of ZrO_2-aquasols (Zirconotrast) in rats

Introduction:

Zirconium dioxide-aquasols (Zirconotrast) and ^{95}Zr incorporated Radiozirconotrast are chosen as test colloids for long term animal studies in order to study their radiation and foreign body effects for comparison to those with thorotrast.

Both to estimate the radiation dose as well as the foreign body effect due to radioactive and non-radioactive colloids, their distribution data in the organs of interest, for example organs of RES, are required.

The organ distribution of non-radioactive ZrO_2-aquasol in rats is estimated by photon-activation of natural ^{90}Zr in the dried organ samples.

Methods and materials:

Female rats (Wistar) were used as experimental animals. The ZrO_2-aquasol, as recently described (EURATOM annual report Biological, Health protection, 1973), was injected in various concentrations of 60, 120, 300 and 600 µl, respectively per animal corresponding to the amount of thorotrast applied in the already started long term study.

In order to determine the distribution of ZrO_2 as a function of time, 10 animals from each concentration group were sacrificed after 1, 10, 20, 50 and 100 days from the administration day.

Bone marrow samples were withdrawn from the femurs.

For the purpose of preparing the samples for irradiation, liver, spleen and lung were frozen and then vacuum evaporated. Aliquots of this thoroughly homogenized material were irradiated in the photon beam of the LINAC of the Bundesanstalt für Materialprüfung, Berlin. Seven organ samples

plus one standard were put in a circular sample holder and irradiated simultaneously for 15 to 30 min. The photon activated samples were measured for ^{89}Zr at least 1 to 1.5 half-lives after irradiation in a NaI(Tl) gamma ray spectrometer.

Results:

The distribution pattern of ZrO_2 in liver plus spleen proved to be significantly dependent on the concentration of the applied colloid and constant during the observation period from 1 to 100 days after injection. 70-75% of the originally administered ZrO_2 is retained in liver and spleen of the 60 µl group and 38-45% in the 600 µl group respectively. The distribution in lung and bone marrow appears to be independent of the applied amount of the colloid. The values in the lung vary from 0.2 to 0.5% of ZrO_2 injected.

The size distribution of the aggregates in liver and spleen does not show any significant variation with time from 10 to 100 days after injection.

Contractant de la Commission:

COMMISSARIAT A L'ENERGIE ATOMIQUE

N° du contrat:

100 - 72 - 1 - BIAF

Chef du Groupe de Recherches:

Dr. J. LAFUMA

Thème général du contrat:

Toxicologie de certains éléments chez les animaux et chez l'homme.

L'année 1975 a permis de dissocier dans l'induction des cancers deux mécanismes: l'un local, l'autre général.

Elle a permis de montrer que l'appaition d'un cancer était la conséquence de deux données:

- l'état de l'animal - d'origine génétique -
- les modalités de l'irradiation.

On peut, dès aujourd'hui, affirmer que l'irradiation interne ne fait qu'accélérer des processus naturels et que, les cancers dus aux rayonnements n'apparaitraient que dans les tranches d'âge les plus avancées de la population, phénomène que l'on a observé chez les fumeurs.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques:

- Chef du projet: Dr. J. LAFUMA
- Collaborateurs scientifiques: Mme W. SKUPINSKI
  M. H. SCHORN
  M. W. MÜLLER
  Melle M. MORIN

Titre du projet: Action toxique des radioisotopes.

1/ ELEMENTS INHALES.

1.1 ACTINIDES

En 1975, se sont terminées de nombreuses séries expérimentales sur l'action toxique des radioéléments émetteurs α inhalés. On dispose aujourd'hui de données dosimétriques complètes sur un millier de rats contaminés avec des actinides, (Pu-239, Pu-238, Am-241, Cm-244, Th-227)

Il ne manque actuellement que 20 examens anatomo-pathologiques.

On a observé les cancers suivants:

Poumons: 235 dont 110 d'origine bronchique
111 d'origine Pneumocytes II
14 sarcomes

OS: 41

Tissus hématopoiétiques: 14

Foie: 2

Rein + vessie : 7

Autres tissus: 32 dont 9 épithéliomes cutanés
6 carcinomes mammaires

Au niveau des poumons, on a observé les lésions suivantes:

- série bronchogénique: Cancers 110
  papillomes inversés bénins: 12
  adénomes à cellules ciliées: 11
  métaplasies épidermoïdes : 62

- série "pneumocytes II" : cancers 111
adénomes bénins 54
adénomatose 43

Si l'on étudie les histogrammes de répartition dans le temps des différentes lésions, on observe:
A/ pour les"Pneumocytes II": une succession adénomatose-adénomes-cancers,
B/ pour les"bronchogéniques":-un histogramme des cancers comparable à celui des cancers à Pneumocytes II mais plus précoce
-un histogramme du papillome inversé bénin très particulier et un histogramme à deux composantes pour les métaplasies épidermoïdes. L'une des composantes semble liée à la tumeur bénigne, l'autre aux cancers.

1.2 EMETTEURS β (Ce-141, Ce-144)

On dispose de données dosimétriques sur près de 300 rats. On a observé: 25 cancers dont: 14 bronchogéniques
4 Pneumocytes II
7 sarcomes
9 tumeurs bénignes
21 lésions type métaplasies ou adénomatoses.

De plus, on a observé: 4 sarcomes des tissus hématopoïétiques.

II/ INJECTIONS LOCALES D'EMETTEURS β (Hydroxyde de Ce-144).

II.1 Intramusculaires (150 rats)

On a déjà observé:
- 97 sarcomes locaux dont:
  - 16 fibrosarcomes,
  - 11 rhabdomyosarcomes,
  - 21 ostéosarcomes d'origine osseuse,
  - 15 angiosarcomes,
  - 19 pericytomes etc...

De plus, on a observé:
- 2 épithelioma cutanés situés près du point d'injection.

La durée de vie de tous les animaux est raccourcie qu'ils présentent ou non un cancer " in situ".

De plus, des études sur la prolifération tumorale ont été menées en collaboration avec l'I.C.I.G. (Villejuif, Pr. MATHE).

Elles ont montré que le temps de doublement dépendait à la fois du type histologique mais aussi du point de départ.

Enfin, on a cherché systématiquement les métastases pulmonaires: 60% des animaux en présentent. Ce phénomène est indépendant du types histologique et de la taille des tumeurs. Il est probablement lié à l'individu lui-même et est d'apparition extrêmement précoce.

II.2 Injection dans un sinus de la face.(Collaboration avec l'.I.C.I.G. et le Pr. JASMIN):

12 rats ont été contaminés avec du Cerium-144.

On a observé 8 cancers locaux. Tous ces cancers étaient des carcinomes épidermoïdes dont aucun n'a métastasé. Il faut rapprocher ce résultat des observations faites avec les êtres humains contaminés avec le Ra-226.

Dans l'ensemble du squelette, on observe des ostéosarcomes, sauf au niveau de la face où on observe que des carcinomes.

III/ ETUDES POURSUIVIES OU LANCEES en 1975.

III.1 Toxicité des actinides.

400 rats ont été contaminés avec des faibles doses d'AM-241.

On a observé chez ces rats l'apparition d'une bronchite chronique infectieuse et on va étudier l'action de ce co-facteur (fréquent chez l'homme) sur l'apparition des cancers.

III.2 Oxyde de Cerium stable.

72 rats ont inhalé une quantité de Cerium stable comparable à celle inhalée par les animaux contaminés au Ce-141, produit d'activation. On étudie leur pathologie générale.

LES PHENOMENES QUI INTERVIENNENT DANS L'APPARITION DES CANCERS "RADIOINDUITS"

I/ Il est admis aujourd'hui que le phénomène principal intervenant dans l'apparition des cancers "radio-induits" est la transformation sous l'effet des rayonnements d'une ou de plusieurs cellules qui passent de l'état "normal" à l'état"cancéreux".

On admet aussi que, si les mécanismes de surveillance n'ont plus une action correcte, le processus cancéreux va se développer entraînant la mort de l'animal.

Cette façon d'aborder le problème est très probablement exacte pour les cancers induits par les virus, mais les résultats de toutes les expériences pratiquées dans divers laboratoires du C.E.A. montrent que cette conception est surement erronée pour les cancers "radio-induits".

II/ Cancers pulmonaires.

II.1. L'obtention chez le rat de cancers pulmonaires, à une fréquence comparable à celle observée pour des doses cumulées semblables, chez les mineurs d'Uranium, a posé un premier problème:

- L'apparition des tumeurs chez le rat étant beaucoup plus rapide que chez l'homme, le résultat pouvait s'interpréter de deux façons: ou bien les phénomènes à l'échelle du tissu cancéreux étaient proportionnels à la durée de la vie, ou bien, si les cinétiques cellulaires étaient comparables, il devait exister un temps de latence caractéristique de l'espèce et dont la durée, pour une même agression, dépendait du mécanisme de contrôle de la durée de vie de l'espèce.

L'expérience a montré que c'était la deuxième hypothèse qui était la bonne.

II.2. L'examen anatomo-pathologique systématique des poumons a montré l'existence avant l'apparition des cancers, de modifications du tissu pulmonaire -proliférations cellulaires anormales et tumeurs "bénignes" -.

Il existe une sorte de "séquence" dans l'apparition des lésions. Ces lésions primitives sont d'ailleurs les mêmes que celles que l'on observe chez les animaux témoins âgés.

II/3. L'étude de la relation entre les doses absorbées et l'intervalle de temps au bout duquel apparaissent les différentes lésions confirment l'interprétation cinétique des phénomènes.

L'irradiation ne fait qu'accélérer une évolution qui se fait spontanément à vitesse plus lente en fonction de l'âge.

II/4. L'étude des greffes de fragments d'organe prélevés, soit en phase de latence, soit en fonction de l'âge chez les témoins confirme également cette interprétation du phénomène.

II/5. Il n'y a pas de corrélation stricte entre la dose tissulaire et la fréquence relative des cancers. Celle-ci dépend plus des caractéristiques génétiques de la souche que de la topographie de l'irradiation.

III/ Cancers des autres organes.

Les expériences pratiquées à faible niveau avec des émetteurs α inhalés dont une fraction irradie les autres organes donnent le résultat suivant: une augmentation de tous les cancers est observée. Mais l'augmentation des cancers d'un organe est d'autant plus élevée que la fréquence de ce type, observée chez les témoins, est plus forte.

IV/ Mécanismes d'induction des cancers radioinduits.

L'hypothèse qui permet le mieux d'expliquer tous les résultats est la suivante.

- Chaque tissu dispose d'un temps limite pendant lequel sa structure restera normale. Ce temps est variable d'un animal à l'autre, mais, pour la plupart des animaux, il est supérieur, pour un tissu donné, à la durée de la vie. A la fin de la vie structurée du tissu, le cancer apparaît.

Chaque tissu ayant son "temps de vie structurelle normale", un animal se caractérise par autant de "temps de vie structurelle normale " qu'il y a de tissus. Il a donc son propre potentiel d'apparition des cancers. Dans une souche donnée, on observera donc tous les cancers, mais certains avec une fréquence plus élevée.

Ce "temps de vie structurelle normale" doit être sous la dépendance d'une double commande, l'une locale, l'autre centrale, liée au "contrôle" de l'évolution de la vie de l'animal.

L'irradiation ne fait qu'accélérer les phénomènes.

V/ L'action des faibles doses.

Cette conception des phénomènes peut permettre d'aborder l'action des faibles doses.

V.I. Le problème du seuil.

Si le processus qui aboutit à l'apparition d'un cancer existe dans tous les tissus de tous les individus et que l'irradiation ne fait qu'accélérer son évolution, il est évident qu'il ne peut y avoir de seuil.

Raccourcir d'une minute, d'une heure ou même d'un jour le temps nécessaire pour que la tumeur débute n'a pas de sens médical -surtout si l'individu est mort pour d'autres raisons dix années auparavant-.

Il existe donc un seuil pratique qui est la dose nécessaire pour que le raccourcissement du temps de latence soit de l'ordre de grandeur des durées minimales qui ont un sens dans l'estimation de l'espérance de vie humaine (mois ou années).

V.II. La fréquence d'apparition des cancers.

L'augmentation de la fréquence d'apparition des cancers n'est que la conséquence du raccourcissement du temps de latence. Elle peut s'estimer si l'on connaît la relation entre celui-ci et la dose absorbée.

Si l'on admet que la répartition des temps de latence des témoins est une fonction de type gaussien comparable à celle de la répartition des temps de survie des animaux, on peut en combinant ces deux fonctions avec la relation entre la dose absorbée et le temps de latence, obtenir une relation entre la fréquence d'apparition des cancers et la dose absorbée.

Cette relation est de type sigmoïde et se caractérise par une diminution très brutale de la fréquence lorsque la dose descend en dessous d'une dizaine de rads α pour les cancers pulmonaires provoqués par l'inhalation d'actinides.

cancers provoqués par l'inhalation d'actinides.

VI. CONCLUSION.

Les études sur les mécanismes interviennent dans l'apparition des cancers radio-induits pourraient, si l'effort entrepris se poursuit pendant deux ou trois ans, permettre d'une part de comprendre toutes les étapes qui aboutissent à l'apparition de ces cancers et, d'autre part, de fixer avec une grande précision la relation entre l'effet et les faibles doses.

PUBLICATIONS 1975.

- Action toxique des radio-isotopes.

LAFUMA J.
Comportement biologique du Plutonium-239
Conférence à la Société Belge de Radioprotection -
Bruxelles - 30 Mai 1975

LAFUMA J.
Les faibles doses de rayonnements ionisants en radiobiologie et en radioprotection.
Séminaire de Radiobiologie de la Sté Fse de Radioprotection
29/30 Avril 1975

LAFUMA J.
Biologie du Plutonium et des transplutoniens
Revue Générale Nucléaire, 1975, 1 , 2, 119-122 - 6 réf,

MULLER W.A., NENOT J.C., DABURON M.L., LAFUMA J.
Metabolic and dosimetric studies after inhalation of ^{227}Th in rats with regard to the Risk of lung and bone tumors.
Rad. and Environm. Biophys. 11, 309-318 (1975)

MORIN M., NENOT J.C., MASSE R., NOLIBE D., METIVIER H., LAFUMA J.
Etude expérimentale de l'action des radioéléments émetteurs alpha inhalés. Raccourcissement de la durée de la vie et induction de cancers. Influence de la dose totale, du débit de dose, de l'étalement de la dose dans le temps, de la répartition spatiale de la dose.
Colloque international sur les Effets biologiques des rayonnements de faible intensité du point de vue de la protection de l'homme et de l'environnement.CHICAGO - 3/7 Novembre 1975.

CHAMEAUD J., PERRAUD R., MASSE R., NENOT J.C., LAFUMA J.
Cancers du poumon provoqués chez le rat par le radon et ses descendants à diverses concentrations. Comparaison de la relation dose-effet chez l'homme et chez l'animal.
CHICAGO - 3/7 Novembre 1975

SKUPINSKI W., MASSE R., LAFUMA J.
Etude expérimentale de l'action des deux émetteurs beta inhalés: Cerium-144 et Cerium-141. Etude du raccourcissement de la durée de la vie et l'induction des cancers. Rôle de la dose. Influence de l'entraîneur.
CHICAGO - 3/7 Novembre 1975

Résultats du projet n° 2

Chef du projet et collaborateurs scientifiques:

- Chef du projet: M. R. BATTI
- Collaborateur scientifique: M. R. BATTI

Titre du projet: Evaluation du risque mercuriel

Des mesures de la contamination du milieu par le mercure et le methyl-mercure ont été pratiquées sur de nombreux échantillons. Les résultats obtenus ont été introduits dans des modèles permettant d'évaluer le risque. Données numériques et évolution des risques ont été exposés en détail dans les publications de la liste ci-jointe.

PUBLICATIONS 1975

- Evaluation du risque mercuriel.

MAGNAVAL R., BATTI R., BOUVILLE A.
Evaluation de la charge corporelle en mercure par simulation numérique.
-Progrès récents dans l'évaluation des effets de la pollution de l'environnement sur la santé. PARIS - 24/28 Juin 1974

MAGNAVAL R., BATTI R., THIESSARD J.
Methylmercury effect on Rat Liver Mitochondrial deshydrogenases "Experentia"

MAGNAVAL R., BATTI R., BITTEL R., BOUVILLE A., GUEZENGAR J.M.
Evaluation par simulation numérique du risque d'intoxication mercurielle associé à la consommation de thon.
European Society for Nuclear Methods in Agriculture.
Cadarache - 8/12 Septembre 1975

BATTI R., MAGNAVAL R., LAMY G. LAFUMA J.
Proceedings problems of the contamination of man and his environment by mercury and cadmium
EUR. 5075

BATTI R., MAGNAVAL R., LANZOLA E.
Methylmercury in river sediments
CHEMOSPHERE 1975, Vol. n° 4 - n° 1

MAGNAVAL R., BATTI R., BITTEL R., BOUVILLE A., GUEZENGAR J.M.
Variation de la charge corporelle d'un toxique en fonction des habitudes diététiques. Evaluation par simulation numérique du risque d'intoxication mercurielle associé à la consommation de poisson.
Colloque international CENECA - PARIS - 26, 27, 28 Février 75.

Contractor:	National Radiological Protection Board
Contract No.:	132-74-1 BIO UK
Head of Research Team:	Dr. G. W. Dolphin
General Subject of Contract:	Binding of actinides to mammalian proteins

Studies have been made of the interactions between plutonium dioxide particles and serum proteins of rats. The experiments were performed in vivo. Plutonium dioxide particles of 4 nm diameter or less behave similarly to soluble plutonium citrate in terms of retention and excretion.

Having shown in vitro that humic acid and fulvic acid have an affinity for plutonium, plutonium-bearing rats were treated with both substances in an effort to stimulate plutonium excretion from liver. The results showed that humic acid in fact caused increased retention of plutonium in liver. Fulvic acid had no influence upon plutonium excretion.

Results of Project No. 1

Head of Project and Scientific Staff: Dr. D. S. Popplewell
Dr. B. W. Loveless

Title of Project: Binding of actinides to mammalian proteins

Suspensions of sub-micron sized particles of plutonium dioxide were injected intravenously into male Wister rats. The plutonium contents of certain organs were measured at various time intervals. Particles having diameters greater than 25 nm behaved as might be expected for a circulating colloid, with most of the activity being found in the liver and spleen. However, particles less than 4nm diameter showed a tissue distribution similar to that of plutonium citrate, i.e. most of the activity became associated with the skeleton. Plutonium when injected as 4 nm particles diameter or less was excreted in the urine more rapidly than plutonium citrate during the first hour after injection, but thereafter behaved as does plutonium citrate. Gel permeation chromatography of serum samples from rats injected with the 4 nm particles gave a binding pattern identical to that of plutonium citrate. No plutonium dioxide particles were detectable in serum taken from rats 5 minutes after injection. It is assumed that the particles were bound rapidly by serum transferrin and citrate, and the increased initial excretion took place within the first few minutes of injection.

In the second experiment, female Wister rats were injected intraperitoneally with ultrafiltered plutonium citrate and then at intervals between one and 7 days they were given 5 intraperitoneal injections of humic acid dissolved in 2% trisodium citrate to produce a total injected dose of 2.8 g per kg. Control animals received tri-sodium citrate only. All animals were killed on the tenth day.

The results (see table) show significantly increased amounts of plutonium in the liver, spleen and kidney relative to controls, thus suggesting that the substance caused a retention of plutonium that would otherwise have been excreted. One hypothesis is that the humic acid had gained access to the lysosomes, complexed the

plutonium, but had not been excreted from the cells as fast as from control animals.

The experiment was repeated using fulvic acid, a substance similar to humic acid which is more soluble under the acid conditions thought to exist in the phagolysosome. However, fulvic acid did not influence either excretion or retention of plutonium.

Percentage of injected plutonium remaining in tissues after 9 days of humic acid treatment (7 rats in each group)

Tissue	Humic-treated	Control	P value
Liver	23.8 ± 2	14.8 ± 0.7	<0.001
Spleen	0.79 ± 0.02	0.36 ± 0.01	<0.001
Kidneys (2)	1.72 ± 0.05	1.05 ± 0.02	<0.001
Tibiae-fibulae	5.71 ± 0.2	6.16 ± 0.2	<0.1
Remaining carcass	42.7 ± 0.9	43.5 ± 0.7	n.s.

Significance of differences (P values) determined by student's t test.

Contractor: United Kingdom Atomic Energy Authority,
Atomic Energy Research Establishment, Harwell.

Contract No.: 076-74-1 PSTUK

Head of research team: A. Morgan (Project No. 1)
A.C. Chamberlain (Project No. 2)

General subject of contract: RADIOACTIVE AEROSOLS

Project 1. Uptake and clearance of inhaled radioactive aerosols

The method for observing the regional deposition of particles in the lower respiratory tract by externally observing γ-emitting inhaled particles and described in the Euratom Report, 1974 (EUR 5332 d-e-f-i-n p479) has been refined. Most significantly, the single detector viewing the stomach region to correct for activity removed to the gastro-intestinal (G.I.) tract has been replaced by a pair of coaxial detectors. These allowed simultaneous measurements of the abdomen with those made of the chest and on the same side of the subject. In this way a better correction was obtained. In order to avoid some of the statistical problems associated with a small number of subjects, a large number of volunteers have been recruited. Relevant background information has been obtained for each volunteer which should greatly improve the interpretation of the results.

Project 2. Assessment of internal contamination with americium-241

Study of the behaviour of americium-241 in eight men

Techniques of body radioactivity measurement have been employed to investigate the retention and distribution of americium-241 in seven subjects who during 1971 were accidentally exposed to airborne contamination in the oxide form. An eighth subject, who acquired a burden on some unknown occasion between 1962 and 1971, is also being studied. Serial measurements of whole body content have been made using arrays of thallium activated sodium iodide scintillators to detect the 60 keV gamma radiation, while the redistribution of the material from its initial site in the lungs is being followed with individual detectors viewing selected anatomical regions.

Calibration of detectors used for the assessment of americium-241 in lungs

Conventional methods of calibration, employing activity incorporated into phantoms, may not be reliable for the assessment of lung contamination with 60 keV gamma-ray emitters if an accuracy of ± 30 per cent or better is envisaged, unless the phantom is realistic anatomically and account is taken of the potential dependence of response on the subject's physique. An alternative approach is the administration to volunteers of short-lived radioactive aerosols emitting radiation of similar energy. A technique has been developed at Harwell for producing motor exhaust aerosols labelled with lead-203 for use in studies of the metabolism of exhaust lead in humans. Lead-203 emits X-radiation at 73 keV, similar in energy to the 60 keV gamma-rays of americium-241, and also gamma-rays at 279 keV which enable the amount deposited in the subject's lungs to be determined independently using established techniques of whole body counting. Detection of the 73 keV X-rays with counters viewing the chest will enable calibration data for americium-241 in lungs to be derived, and data for subjects covering a range of body weights have been accumulated.

Results of Project No.: 1

Head of Project and scientific staff: A. Morgan
N. Foord

Title of Project: DEPOSITION AND CLEARANCE OF INHALED MONODISPERSE PARTICLES IN THE HUMAN RESPIRATORY TRACT

A considerable fall was found in the variation with time following inhalation of the correction necessary to account for the activity cleared from the lung to the G.I. tract after the installation of a pair of coaxial detectors in line with the chest detectors and viewing the abdomen. A further reduction was found when the right side of the subject only was measured. A series of measurements of the chest-to-gut region counting rates was made following the ingestion of radioactive particles by seven volunteers. Three of these volunteers subsequently took part in inhalation experiments and the results are given in Table 1. The observed retention of particles after 24 hours was corrected in two ways. Firstly, a correction was applied based upon the results of their own ingestion experiment and secondly a correction, based upon the average of the results of the ingestion experiments with all seven volunteers, was used. It is clear from this that if results relating to an individual are required, a correction based upon an ingestion experiment with that individual is necessary.

Table 1

RETENTION OF RADIOACTIVITY IN THE RIGHT CHEST
24 HOURS AFTER ADMINISTRATION OF LABELLED PARTICLES

Subject	Nominal particle size (μm)	Right chest retention		
		No Correction (%)	Individual Correction (%)	Average Correction (%)
1	5	75	69	73
2	5	71	68	67
3	5	44	43	35
1	7.5	51	31	44
3	10	24	20	-33

In order that the results are more representative, a larger group of volunteers was recruited from which groups may be selected for comparison. Following an advertising campaign, every person who responded was interviewed and completed a questionnaire which gave details of any respiratory ailments past and present, smoking habits and exposure to dusty environments. A lung function test was also carried out which measured lung capacity and flow rates from a forced expiration. This information will be valuable in selecting the groups and for interpreting the deposition results for any individual.

Publication

FOORD, N., BLACK, A., WALSH, M. Pulmonary deposition of inhaled particles with diameter in the range 2.5 - 7.5 μm. Presented at the 4th International Symposium on Inhaled Particles and Vapours, Edinburgh, organised by The British Occupational Hygiene Society, 1975.

Results of Project No.: 2

Head of Project and scientific staff: D. Newton
Miss F.A. Fry
(until June 1975)
B.T. Taylor
M.C. Eagle

Title of Project: ASSESSMENT OF INTERNAL CONTAMINATION WITH AMERICIUM-241

Study of the behaviour of americium-241 in eight men

In the seven individuals whose contamination was acquired during 1971, investigations covering the approximate period 200-1600 days after intake have shown (i) no evidence of any reduction in whole-body content, except possibly by 15% and 30% in two instances, (ii) a reduction in the lung burden with biological half-lives in the range 500 days to > 2000 days for the various subjects, and (iii) translocation of americium-241 to liver and bone. The latter is illustrated for Subject CR (total body content 59 nCi) in Figure 1, which shows the counting rates recorded by collimated detectors viewing selected bony regions. The increases have been most marked in the long bones of the legs, with much smaller increments to activity in the sacrum and no detectable change for the head. Figure 2 shows that the rate of accumulation in the leg bones of this subject is consistent with the rate of loss from the lungs, which was shown to occur with a biological half-life of 884 $\pm$ 117 days. By contrast, in the case of Subject MB, whose contamination may well have been acquired in a quite different physico-chemical form, there has been no detectable translocation of americium-241 from the chest.

Calibration of detectors used for the assessment of americium-241 in lungs

Immediately following each subject's exposure to a lead-203 aerosol the response from the 73 keV X-rays was recorded using a phoswich detector positioned centrally in contact with the frontal surfaces of the chest. The detector consisted of a sodium iodide (thallium activated) crystal, 20 cm dia. and 3 mm thick, coupled to a caesium iodide (thallium activated) crystal 20 cm dia. and 5 cm thick, which was viewed by an 18 cm dia. photomultiplier tube; pulse shape discrimination methods were employed so that X-ray interactions in the sodium iodide element were preferentially

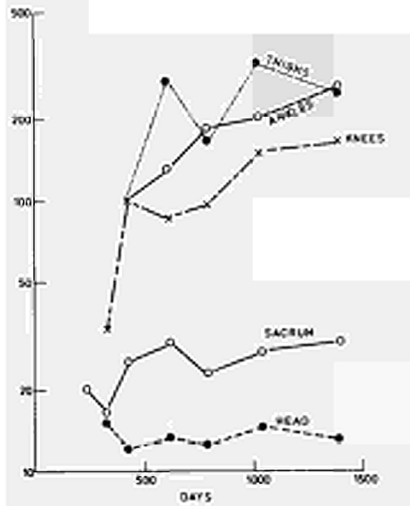


Figure 1

Counting rates from a collimated scintillation counter viewing various bony regions. (Subject CR; values for thighs, knees and ankles have been normalised to 100 at 427 days)

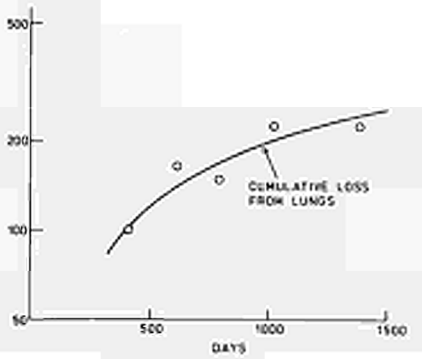


Figure 2

Plotted points: mean of data of Figure 1 for thighs, ankles and knees. Fitted curve: calculated cumulative loss of americium-241 from lungs with biological half-life 884 days, normalised to 100 at 427 days.

recorded. In Figure 3 are shown the detection efficiencies (counts per minute per nCi of lead-203 deposited in lungs) for six subjects plotted against the ratio weight/height, which is a measure of the average cross sectional area of the body. As expected, there is a trend towards lower efficiencies in larger subjects. The relative absorption of 60 keV and 74 keV radiation in tissue substitutes is currently being studied and the results will enable detection efficiencies for americium-241 in lungs to be derived from the data of Figure 3.

Publication

FRY, Miss F.A. Long term retention of americium-241 following accidental inhalation. Health Physics (in the press).

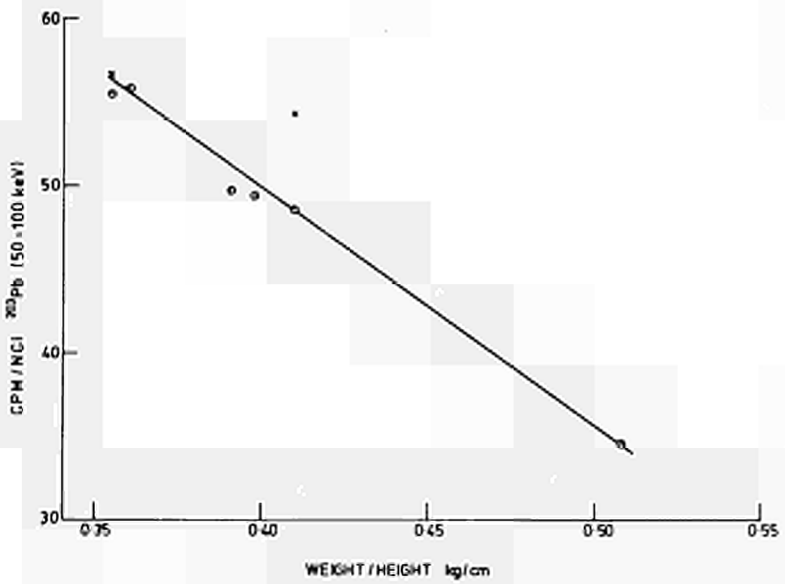


Figure 3

Detection efficiencies (counts per minute per nCi present in lungs) as a function of the ratio weight/height for subjects exposed to lead-203 released in a wind tunnel (⊙) or inhaling lead-203 from a closed container (X).

Contracting party of the Commission
EUROPEAN LATE EFFECTS PROJECT GROUP (EULEP)
Contract number : 092 - 72 - 1 BIO C
Chief of the research group : C.F. HOLLANDER
General object of the contract :
PERFORMANCE OF A CO-OPERATIVE RESEARCH PROJECT ON LATE SOMATIC EFFECTS OF IONIZING RADIATION IN MAMMALIAN ORGANISMS.

EULEP has pursued its efforts during the year 1975 on

a) standardization of experimental conditions in the participating institutions.
b) coordination of the planning and performance of on going research projects in the area of radiation late effects, and
c) on execution of specific cooperative projects on carcinogenesis, on dysplasic and dystrophic lesions and on the toxicity of radioisotopes.

STANDARDIZATION COMMITTEES

B-1. Standardization of radiation dosimetry
B-2. Standardization of conditions for animal experimentation
B-3. Standardization of histopathology
B-4. Standardization of laboratory methods.

SPECIFIC COOPERATIVE PROJECTS

B-5. In the field of carcinogenesis
B-6. In the field of the non-neoplastic changes
B-7. In the field of internal radioisotope toxicity.

Results of project N° 1
Head of the project : J.J. BROERSE
Title of the project : COMMITTEE ON DOSIMETRY STANDARDIZATION

The European Neutron Dosimetry Intercomparison Project (ENDIP) has been conducted over the period March to December 1975. The intercomparison at the Institut für Strahlenschutz, GSF, München/Neuherberg has been performed for free-in-air conditions for monoenergetic neutrons with energies of 0.67, 2.1, 5.5 and 15.5 MeV and for the mixed n-γ radiation from ^{252}Cf. At the Radiobiological Institute TNO, Rijswijk measurements of neutron and gamma ray absorbed dose in a phantom have been performed by twelve groups for collimated neutron beams with energies of 5.5 and 15 MeV produced by the d-D and d-T reaction respectively. Details of the experimental arrangements employed at Neuherberg and Rijswijk can be obtained from the chairman upon request. The evaluation of the neutron dosimetry intercomparison will be performed in the near future

Preparations are made for a third X-ray dosimetry intercomparison program, which will be performed in the spring of 1976. This intercomparison is considered to be essential in view of the joining of new groups in the past years. As a pre-requisite for the intercomparison, the new groups are requested to perform their irradiations and dosimetry according to the protocol for EULEP X-ray dosimetry.

Dosimetry studies have been started for partial-body irradiations, performed to investigate the response of specific organs. Various exposure arrangements employed for irradiation of lungs in experimental animals at London, Louvain, Mol, Oxford, Rijswijk and Warsaw will be compared.

Publications

J.J. BROERSE and K.J. PUITE. The usefulness of intercomparison studies for the improvement of X-ray dosimetry. Phys. Med. Biol. 19, 732-734 (1974).

K.J. PUITE, G. SCARPA and J.J. BROERSE. X-ray dose and dose distribution intercomparisons using mailed LiF and BeO thermoluminescent dosimeters. In : Proc. 4th Int. Conf. on Luminescence Dosimetry, Krakow, p. 963-976 (1975).

Results of project N° 2
Head of the project : A. DUNJIC
Title of the project : COMMITTEE ON LABORATORY ANIMALS STANDARDIZATION (CLAS)

Longevity studies

The longevity studies, which are now going on for 2 years, involve 15 EULEP laboratories. Two new series of control animals were started in 11 laboratories during 1975. In addition four laboratories commenced new experimental series for the study of bacteriological and nutritional aspects, including biotyping assays.

An improved computer version of life table technique is in operation this year. Recently a new program on mortality rates was initiated following the procedure recommended by Kimball (Biometrics 1960, 16, 505-521). Both computer programs were used to analyze previous or preliminary longevity data from five EULEP laboratories.

LD50/30 studies

A progress report (prepared by Metalli) appeared in the July issue of EULEP Newsletter (N° 8 pp 13-24). It summarizes the experimental results for mice. A second progress report is in preparation for data from rats. Determinations of the LD50/30 is continued voluntarily in several EULEP laboratories.

Other activities

The inventory of strain of animals in EULEP laboratories has not changed sufficiently to warrant a new listing.

Only eight of eighteen EULEP laboratories appear in the recent third edition of the International Index of Laboratory Animals (MRC 1975). Address codes are : Cnb (Mol), Bts (London), H (Harwell), Rij (Rijswijk), Rind (Sundbyberg), Ulg (Liège B), Wsr (Louvain) and Ztn (Ulm).

A recent inquiry by CLAS among EULEP laboratories provided an evaluation of expenditures for long-term studies. In six EULEP laboratories the actual costs range from 0.37 to 0.88 BF (mice) and from 1.00 to 1.92 BF (rat) per animal and day.

About 832 bibliographic references on late radiation effects were processed by an appropriate computer program. These concerned with longevity data are selected for distribution among EULEP members.

Results of project N° 3
Head of the project : W. GOSSNER
Title of the project : COMMITTEE ON PATHOLOGY STANDARDIZATION

In 1975 two workshops have taken place in Munich. The first workshop was held on April 5-6, 1975 with the main topic "Neoplastic and non-neoplastic lesions of nervous system".

Twenty-one selected cases of tumors of the nervous system in rats and rabbits and in addition 6 cases of lesions of the eye in dogs were presented and discussed. These cases included examples of the following lesions :
Oligodendroglioma, Astrocytoma, Glioblastoma multiforme, Malignant schwannoma, Meningeal melanoma, Granular cell tumor, Esthesioneuro-epithelioma, Malignant melanoma of the eye, Lymphosarcoma of the eye, Sclerosing pseudotumor of the eye, Mycotic ophtalmitis.

Lectures were given by Dr J.W. Hopewell on "Hormone dependence of experimental inducted gliomas",
Dr A.J. van der Kogel on "Pathogenesis and morphological aspects of irradiation damage at different levels of the spinal column of the rat", and Dr J.D. Burek on "Pathological features in spinal cords from paralysed rats". Dr Stavrou (University of Munich) reported on the demonstration of the glial-specific protein S-100 in brain tumors.

In the final discussion on histogenesis and classification of the neoplastic lesions of the nervous system several proposals have been presented by the participating experts. The classification adopted is similar to the histological classification of central nervous system tumors published in R. Escourolle and J. Poirier, Manual of Basic Neuropathology, Philadelphia : Saunders, 1973, p. 40.

The second workshop was held on November 23-24, 1975 with the main topic "Neoplastic and non-neoplastic lesions of the cardio-vascular system and soft tissues".

Fourty-five selected cases of lesions of the cardio-vascular system and soft tissues in mice, mastomys, rats and rabbits were presented and discussed. These cases included examples of the following lesions of the cardio-vascular system :

Tumors : Benign hemangioma (cavernous, capillary, sclerosing), Malignant hemangioendothelioma and hemangiopericytoma, Kaposi-like tumor.
Other lesions : Arteriosclerosis, Periarteriitis nodosa, Radiation-induced vascular lesion, Endomyocarditis, Endomyocardial disease, Calcification of heart muscle.
The cases of soft tissue tumors included examples of : Undifferentiated sarcoma, Pleomorphic sarcoma, Malignant fibrous histiocytoma, Spindle cell sarcoma (Fibrosarcoma), Rhabdomyosarcoma, Leiomyosarcoma, Malignant mesothelioma, Malignant multicomponent tumor, Mastocytoma.

The following nomenclature of neoplasms of the soft tissue and vascular system has been discussed :

Undifferentiated sarcoma	without specific
Pleomorphic sarcoma (with or without giant cells)	arrangement of cells
Spindle cell sarcoma (Arrangement of cells in sheats)	
Fibrosarcoma	
(Malignant schwannoma)	
Rhabdomyosarcoma	
Leimyosarcoma	
Fibrous histiocytoma	
Sclerosing hemangioma	
Hemangioma, benign	
Malignant hemangioendothelioma	
Malignant hemangiopericytoma	
Multicomponent (combined tumors) e.g. Mesothelioma	

Comment : So-called Reticulum cell sarcoma, Mastocytoma, Histiocytoma excluded

In addition, in both workshops several problem cases which were sent to the consultation center were presented and discussed.

Publications

W. GÖSSNER, C.F. HOLLANDER, J.R. MAISIN, A. NILSSON . EULEP Pathology Atlas - European Late Effects Project Group - Committee on Pathology Standardization, Workshop October 1971 - Bone tumors in mice and rats.

Results of project N° 4
Head of the project : A. KEYEUX
Title of the project : COMMITTEE FOR CLINICAL PATHOLOGY STANDARDIZATION

The committee cooperated in two projects of standardization and coordination

1. the supply of ALS and ALG which was necessary for the DIMS project (D.W. Van Bekkum)
2. the development and standardization of the EULEP data analysis (A. Dunjic).

The aim pursued by the development and standardization of the EULEP data analysis is to introduce and develop computer processing of the longevity data for intercomparison studies within EULEP laboratories.

Since the beginning of 1974, an improved version of the life table technique is operational. The input data are sorted in increasing order. As output the values of the main longevity parameters are listed following each life table print. A table summarizes again all these parameters after a definite number of population samples has been processed. Further, comparitive plots of death histogram and mortality rates were improved for the ease of data inspection.

Recently a second program on the mortality rates or intensities following the procedure recommanded by Kimball (Biometrics, 1960, 502-521) was set up. This procedure uses non parametric methods for small population samples, in well controlled experiments uncomplicated by losses and age variations. In this program, an estimate of mortality intensity is obtained based on a pre-asigned number of deaths and on the boundaries of the intervals as random variables.

Both computer programs were used extensively for the analysis of previous or preliminary longevity data from five EULEP laboratories. In addition pertinent experimental data available from literature were processed. Thus the performance and restrictions of the standardized procedures could be examined and defined and the possibilities of standardization of longevity data analysis were demonstrated.

Results of project N° 5
Head of the project : H. SEIDEL
Title of the project : COMMITTEE ON CARCINOGENESIS

Leukemia induction (Coggle, Lindop/London and Metalli/Rome)

A study in SAS/4 mice was completed at London. In this strain male mice exhibit twice the incidence of radiation induced leukemia of that of females. It was investigated whether such differences are related to the number of "cells at risk" and their postirradiation cell kinetics. An intensive study on cellular changes in thymus and bone marrow after irradiation with doses of 200 - 600 R indicated no significant difference between the sexes. Both the thymus and the bone marrow show a similar rapid fall in cellularity and an equally rapid recovery to normal values. Changes in postirradiation cellularity patterns including CFUs of the bone marrow over a 15 months period gave very little insight into the mechanism of radiation leukemogenesis.

In Rome the studies with radiation chimeras are still in progress. Donor cells are treated with 4 x 150 R alone or in combination with urethane. The first lymphomas have been observed and the overall mortality has now reached over 50 % ; the end of the experiment is expected during 1976.

Development of immune monitor systems (DIMS group)

The development of suitable monitor tests for the immune system of mice and rats in long term studies is the goal of the activity. Antilymphocyte sera from the horse are used as immunosuppressive agent in order to have a common standard for immuno suppressor groups in the different models of carcinogenesis to be monitored. In mice a number of suitable tests using spleen cells have been established. These include mitogen induced cell proliferation, cell mediated lympholysis and leukocyte migration. Unfortunately, the reliability

of these tests is not entirely satisfactory and they require the killing of the animal. Peripheral blood tests also exhibit considerable variations but a test based on mitogen induced cell proliferation seems to work satisfactorily. A follow up study of individual animals in long term studies will thus be possible. In rats base line values were obtained for mitogen assays in different lymphoid organs in three strains for different age groups.

Liver carcinogenesis (D. Jovanovic)

In this study which is still in progress the liver of rats is irradiated locally by i.v. administration of ^{198}Au colloid. The different groups received a) internal radiation alone, b) radiation and normal horse serum, c) radiation and antilymphocytic serum. The last group must be repeated since the chronic application of ALS was toxic for the rats. Now toxicity studies for ALS were included. The results will be available at the end of 1976.

Bladder carcinogenesis (Bolhuis/Rijswijk)

The studies are still in progress. Tumors have been observed in the rats after methyl-nitroso-urea. Immune parameters are determined according to the DIMS methodology. An overall report is expected during 1976.

Results of project N° 6
Head of the project : W. CALVO and G. GERBER
Title of the project : COMMITTEE ON DYSPLASIA AND DYSTROPHIA QUANTITATIVE AND QUALITATIVE CELL CHANGES

1. *Late effects in irradiated lung*

Biochemical endpoints (Dancewicz, Gerber)

Biochemical changes were studies in lung of rats exposed to 650 R of whole body X-irradiation (Warshaw). Results which are now prepared for publication indicate, in contrast to the results after hemithorax irradiation with 3KR (experiments carried out in Mol), no significant changes in collagen content during the rate phase after whole body exposure. Moreover no inhibitation of fibrinolytic activity but rather an activation was also noted in these animals.

The study of radiation-induced changes in collagen structure revealed the appearance of new, borohydride-reducible components in solutions of irradiated collagen. These components are presumably involved in the formation of new atypical crosslinks, which facilitate the non-reversible aggregation of collagen (Paper in press in Acta Biochim.).

The data on the experiment with 3 KR hemothoracal irradiation have been evaluated and the paper has been sent out for publication (Mol). In summary, the following changes were found. After a slight early decrease, collagen increased during the fibrotic stage. An increase during fibrosis was also seen for DNA, β-glucuronidase, cathepsin, histamine, serotonin and lipid peroxides. Fibrinolytic activity was found depressed at most time points studied.

Techniques were developed to isolate and assay lung surfactant and a new series of experiments has been started using 1 KR hemithorax exposure. Moreover, another experimental series was started in which rats were irradiated and/or

exposed to high concentrations of SO_2 for 2 days. First data are available for the period of 2 weeks and 3 months.

Physiological endpoints (Keyeux, Jovanovic)

Changes in ventilation were tested by comparing ^{133}Xe clearance in the irradiated animals at 500, 1000 and 1500 R and their unirradiated controls. A net reduction of tne regional ventilation per unit of volume was observed in the well and poorly ventilated compartment during the acute phase (about 1 month after irradiation) and only in the poorly ventilated compartment during the late phase (about 1 year after irradiation) for every dose used.

Local changes in pulmonary extravascular water content during the development of radioinduced lung fibrosis were also evaluated after exposure to 500, 1000 and 1500 R. As a consequence of the moving of the laboratory to Brussels, the computer processing of these experimental data has been delayed and therefore final results are not yet available.

Intercomparison project (Lindop, Gerber)

The irradiation of mice for this project should be completed before Christmas 1975. Groups of mice have been irradiated (whole thorax, right or left hemithorax) with either 0.5, 1.0 or 1.5 krad of either 200 kVp X-rays or 15 MeV electrons. The mice are being kept for 2 or 6 months before sacrifice either here at Barts or at Mol. If the animals are sacrificed here their lungs are sent to Mol for biological tests. The first groups of mice have already been killed and it is hoped that the thoracic irradiation of rats will be started after Christmas.

Relationship between cells at risk and incidence of lung tumors (Lindop)

The lung tumor incidence in mice (SAS/4 and C3H) following exposure to external whole body irradiation on its own and in conjunction with the administra-

tion of the carcinogen urethane has been examined. Lung tumor incidence has been found to depend upon the strain of mouse and their age at exposure. The corresponding perturbation of cell kinetics has been investigated using flash labelling with tritiated thymidine. It is proposed that the present knowledge be extended to include the comparative effects following exposure to internal emitters in the lung, e.g. plutonium and high levels of tritium.

Animals that have been fully labelled throughout foetal live with tritiated thymidine are now available for cell kinetic studies. The mice have been infused "in utero" and had a concentration of < 0.5 µCi/gm body weight of tritium at birth. As it is proposed that tritium also be used as damaging agent other animals have been infused with higher doses (> 1 µCi/gm body weight at birth).

Morphological endpoints (Maisin)

In order to determine the RBE of neutrons versus X-rays for the induction of Radiopneumonie, BALB/c male mice of three months of age were irradiated on the thorax with increasing doses of X-rays and of neutrons of 15,35 and 50 MeV. The analysis of the results is in progress.

2. *Late effects of irradiation on the central nervous system as a model for late vascular changes*

The group, consisting of Calvo (Ulm), Maisin and Gerber (Mol), Hopewell (Oxford), Keyeux (Louvain) and Reinhold (Rijswijk) continued its investigations regarding the mechanisms responsible for the development of late radiation damage, as may occur after therapeutic doses of irradiation. The model is the rat brain, the (single) dose of X-rays is 2000 rad. Radiation is performed at Louvain, Mol and Rijswijk and specimen, obtained by serial sacrifice, sent to the other cooperating laboratories. In total, some 50 different determinations are being performed, covering structure and ultrastructure, biochemistry, cell kinetics and circulation physiology. Because the vascular system is generally regarded as one, if not the only, dose limiting factors, the emphasis is placed on factors concerning the vascular system of the rat brain.

The last year was - for most of the investigations - the second year of follow-up after irradiation, and gradually more abnormalities seem to develop during the second year. General aspects are a high incidence of hydrocephalus of the irradiated brains, and a sizable incidence of pituitary tumors in controls as well as irradiated animals. With regard to the cell kinetics in the cortex of the irradiated rat brain, despite careful quantification no essential changes could be observed in the endothelial cells, and oligodendroglia cells of the white and grey matter. The subependymal plate, however, suffers a sizable loss of proliferative activity. Its relation to the development of late, manifest damage is not yet clear, but it may be that mechanisms similar to stem cell depletion are responsible.

Somewhat in contrast to the expectations is the finding that - after the dose of 2000 rad - no significant changes in the ultrastructure or permeability can be demonstrated of the endothelial cells or the basement membrane of the cortex. After higher dosages, damage as well as signs of repair occur, however, in neurons and satellite cells.

With regard to the à vue 3 dimensional structure of the blood vessels of the rat brain, it is becoming apparent that - after a latent period of 15 to 18 months - vascular malformations develop in the white matter, with negligable changes in the grey matter. Recent determinations of the density of the capillary system in the cortex showed, however, an increase of 23 - 35 % while there was no change in the thickness of the cortex. An increase in capillary density would correlate with the findings by the circulation physiologist of the group, that the circulation in the CNS is increased at 15 months post-irradiation. It does, however, not fit with the finding that there is no change in the oxygenation status of the brain, as estimated by NAD(H) fluorescence "in vivo".

During the early period, an increase in uptake of α aminobutyrate (AIB) and a temporary depression in β glucuronidase and cathepsin activity followed by an activation at one month was seen. Somewhat later, acid phosphatase increases. During the intermediate period, DNA and serotonin content and AIB uptake by brain increase whereas AIB uptake by heart and muscle decrease. A fall in sialic content is also noted at this time. During the late phase collagen increases AIB uptake by brain and liver decreases.

When one puts the afore mentioned results together, a picture emerges that requires considerable clarification. Physiological parameters, like cerebral circulation seem to take place without effecting the oxygenation. Increased capillary density develop without obvious endothelial cell proliferation, or ultrastructural changes.

The group will continue to work on the various questions that have arisen and that may indicate the way of how late effects of radiation do develop.

Publications

A.M. OANCEWICZ, A. MAZANOWSKA and G.B. GERBER. Late biochemical changes in the rat lung after hemithorax irradiation. Radiation Res., in press.

G.B. GERBER, H.S. REINHOLD, J. DEROO and B. BESSEMANS. Late effects in the central nervous system. A study of biochemical alterations after local exposure of the rat brain to 2 KRAD. Strahlentherapie, in press.

H.S. REINHOLD, A. KEYEUX, A. DUNJIC, D. JOVANIVIC and J.R. MAISIN. Current Topic in Radiation Research Quarterly, Editors : M. Ebert and A. Howard, Vol. 10, Nos 1 and 2, 1974.

H. REYNERS, E. GIANFELICI de REYNERS, J.M. JADIN and J.R. MAISIN. An ultrastructural quantitative method for the evaluation of the permeability to horseradish peroxidase of cerebral cortex endothelial cells of the rat. Cell Tiss. Res., 157 (1975) 93-99.

H. REYNERS, E. GIANFELICI de REYNERS, J.M. JADIN and J.R. MAISIN. Les citernes subsuperficielles du réticulum endoplasmique et leurs relations avec les pieds astrocytaires apposés sur les neurones dans le cortex cérébral. J. Microsc. Biol. Cell., 23 (1975) 71a.

Results of project N° 7
Head of the project : P.J. LINDOP
B.E. LAMBERT (Tritium and its compounds)
W.A. MULLER (Bone-seeking isotopes - EBONY)
Title of the project : POINT SOURCE EFFECTS COMMITTEE

The work of the Point Source sub-committees was discussed at a full Committee meeting in Rome, in October 1975.

The EBONY project had completed interlaboratory comparisons in mice of different strains, of the distribution of Sr^{90} (1), Ra^{223} (2) and Pu^{239} (3) and has been published in part. The data confirmed that the assay methods were comparable for distribution determinations between participating laboratories ; that some anomalous results were explicable on the exact meaning of anatomical sites, such as "knee joint", "lower end of femur", etc., and did not present a problem as far as future intercomparison studies of distribution, (and subsequently of effects) of incorporated bone seekers were concerned.

The second EBONY-experiment was defined in 1975 and is expected to result in a long-term late effect study with a 5 years (maximum) duration. The first stage of this experiment was formulated and agreed on the occasion of the Meeting of the Point Source Committee and 3rd EBONY-Meeting, Rome and Casaccia 16 - 17 October 1975.

This experiment should be an interstrain-interspecies study carried out with three long-lived bone-seeking radionuclides (^{90}Sr, ^{226}Ra, ^{239}Pu) in at least 6 EULEP institutes. Two incorporation periods (28 days and life-time) and two dosages of each radionuclide should be used : the lower one with a presumptive osteosarcoma incidence significant above controls (ca 10 %), the other one with a rather high incidence (more than 50 %).

This study will not be synchronized, in order to enable each participating institute to incorporate the experiment arbitrarily in its normal program. Thus in addition new partners can join the project at any time.

The Tritium project has continued to study the toxicity of HTO compared with ^{3}H thymidine, when incorporated during foetal life in rodents (rats - Ulm, mice - Barts). Studies of neonatal deaths, and long term effects, as on body weight, lifespan and causes of death are being correlated with the retention of the tritiated compounds in mice, which have been serially sacrificed for biochemical determination of ^{3}H-labelled organic, DNA, and inorganic components of the tissues. Some of these results were reported at an international meeting at Julich, in September 1974 [4, 5]. One somatic effect of interest in relation to the effects of a single dose of wholebody X-rays externally to the developing mouse, has been the observation of a body weight overshoot, i.e. excess weight gain in the lower dose internally irradiated groups. The possible correlation between such an abnormal weight excess α-pituitary-hypothalamic lesion is being further investigated.

Publications

1. G. WALINDER and W.A. MULLER. Concentration of ^{90}Sr and ^{90}Y in various organs in the female mouse. FOA Report C. 40021-A3, 1975.
2. W.A. MULLER et al. Distribution of Ra^{223} in various organs in the mouse. EULEP Newsletter 9, 5-24, 1975.
3. E.R. HUMPHREYS, P. METALLI, A. SEIDEL, Z. SZOT and O. VANDERBORGHT. The distribution of Pu^{239} in several strains of mice - a collaborative experiment. In course of publication.
4. B.E. LAMBERT and M.L. PHIPPS. Some effects of irradiation of mice "in utero" with tritiated compounds. In Proceedings of International Conference on Molecular- and Microdistribution of Radioisotopes and Biological Consequences, Julich, 1975. In course of publication.
5. W. SCHREML and T.M. FLIEDNER. Distribution of tritiated compounds (tritiated thymidine and tritiated water) in the mother-fetus system and its consequences for the radiotoxic effect of tritium. In Proceedings of International Conference on Molecular- and Microdistribution of Radioisotopes and Biological Consequences, Julich, 1975. In course of publication.

Contractor: International Commission on Radiological Protection.

Contract No: 91-73-1 BIOC.

Head of research team: C.G. Stewart, Chairman, ICRP.

General subject of contract: Development of fundamental data on radiation exposures and the establishment of recommendations regarding maximum permissible exposures.

Brief general description of the work carried out:

In 1975 the Commission and its committees met to review the work being performed by ICRP, including the revision of the Commission's basic recommendations. Representatives from a number of organisations, including the Commission of the European Communities, were invited to the meeting.

In 1975 the Commission published its report on Reference Man (ICRP Publication 23). This report is a comprehensive review of those anatomical, physiological and metabolic features of man that are of importance for radiation protection.

In addition to the meeting of the Commission with all its committees, Committees 1 and 4 met to review work being performed by their task groups. These committees also helped the Commission to prepare certain sections of its new basic recommendations, which are expected to be completed in 1977, and which will replace ICRP Publication 9.

ICRP committees and task groups worked on the following topics in 1975:

Biological effects of inhaled radioactive particulates.

The radiosensitivity of the embryo and foetus.

The influence of factors such as LET and protraction of exposure on genetic and somatic hazards.

The quantification of the severity of radiation effects for the purpose of estimating detriment.

Dosimetry of radionuclides within the body (a revision of ICRP Publication 2).

The hazards of radon, thoron and their daughter products.

Respiratory absorption and elimination mechanisms.

Protection of the patient in radiotherapy.

A revision of ICRP Publication 5.

Emergency and accidental exposures.

Radiation protection in uranium mines.

Releases of radioactivity into the environment.

The membership of the Commission and its committees is unchanged from 1974.

GRUPPE BIOLOGIE ISPRA
KOMMISSION DER EUROPÄISCHEN GEMEINSCHAFTEN

BIOLOGY GROUP ISPRA
COMMISSION OF THE EUROPEAN COMMUNITIES

GROUPE DE BIOLOGIE ISPRA
COMMISSION DES COMMUNAUTES EUROPEENNES

Biology Group Ispra-Italy

Collegial Direction:
Executive Secretary : R.CAVALLORO
Head of research Project 1 : A. BERG
Head of research Project 2 : F.CAMPAGNARI
Head of research Project 3 : J.BOOZ

General subject of Contract: Direct participation of the Commission in its established programme

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Brief presentation

The Biology Group Ispra has been run this year under a collegial direction composed of the Executive Secretary and the leaders of the three research projects.

The Group has contributed during 1975 to the research training and education of 9 students and 6 post-graduate students, in the framework of the Scientific and Technical Education Programme of the Commission. Furthermore, three scientists from other EURATOM contracts, have collaborated within the Group.

The research activities, carried out in close connection with various EURATOM Association or Group Contracts, have been developed under the following three principal headings:

1. Environmental contamination and radiation effects.

Investigations on the transfer of various radionuclides (radioiodine, radiozinc and radiostrontium) and of associated pollutants (cadmium) along the food chain were continued, in collaboration with the Association EURATOM-CEA and the Joint Research Centre. On terrestrial systems, the direct contamination of plants by spraying and the behaviour of radionuclides in soils, their transfer to rice and the effect of ionizing radiation on rice seeds, were studied. On aquatic systems an effort as been developed in order to consider the transfer of Zn-65 and Cd in "natural" food chains of various types.

The investigations of chemico-biological interactions were concerned

with the determination of equilibrium concentrations of metals in presence of complex forming species and with the continuation of the studies of metal binding proteins in mammals (in collaboration with the Joint Research Centre) and their extension to fish and plants.

Entomological research was performed as integrated part in the Commission's contractual programme "Radioentomology". Studies were mostly concerned with ionizing radiation effects on insects of economical importance in view of their control in stored products as well as in the field via the sterile male technique.

2. Biochemical studies on DNA damage and DNA repair.

The studies concerned mainly molecular aspects of the radiation injury to DNA and the interaction between the damaged DNA with mammalian enzymes involved in the replication and the repair of the genetic substance. Partially purified DNA polymerases were characterized with respect to their sensibility to natural and semisynthetic antibiotics inhibiting DNA synthesis. The distribution pattern of various DNA polymerase activities extractable from cell nuclei was defined.

Advanced physical methods of structural analysis (ESR, NMR, linear dichroism and birifringence in an electric field) were improved and adjusted to investigate the molecular integrity of the nucleotide material dispersed in aqueous solutions. This activity was carried out in collaboration with the Division of Physics of the J.R.C. at Ispra.

In the analytical and biochemical experiments, large use was made of synthetic polynucleotides which served as experimental model compounds to counterfeit DNA with specific chamical lesions or conformational changes. Recently discovered methods of chemical synthesis and improved procedures of enzymatic condensation of mononucleotides were applied to obtain a diversified and large scale production of the DNA-like polymers. Most of such compounds were prepared under request and then supplied to European laboratories carrying out researches on DNA repair under the sponsorship of the Programme "Radioprotection-Biology" of the C.E.C. This cooperation extended to the following Institutions: Université Libre de Bruxelles, University of Sussex at Brighton, Rijksuniversiteit Leiden, Università di Roma, Università di Pavia.

3. Radiation structure of ionizing radiations in tissue and its relation to their spectral energy deposition in biological structures and to the biological effectiveness.

As in the past this activity was performed as part of the integrated long-term programme of the "European Dosimetry Group" (see sector III, 1. Dosimetry). It comprises two projects:

- Radiation structure in biological material and in model substances: it is the scope of these studies to provide the basic physical data which are needed in dosimetry, microdosimetry and radiobiology, like e.g. W-values of directly and indirectly ionizing radiation, ranges and stopping powers of electrons and ions, track structure of charged particles and their delta-rays in tissue equivalent materials, etc.
- Evaluation of the energy deposition of different types of radiations to small biological volumes and its relation to the corresponding biological effectiveness: energy deposition spectra of neutrons, X-rays and gamma-rays, can provide basic information for the dose dependence of the relative biological effectiveness of different biological end points. Therefore it is the scope of these studies to evaluate energy deposition spectra of X-rays, gamma-rays, fast neutrons, and their secondaries in biological tissue and to apply them for the interpretation of biological effectiveness.

Results of Project No. 1

Head of Project and scientific staff: A.Berg, R.Cavalloro, E.Levi, M.Merlini*, P.Reiniger, P.Scoppa

Title of Project: Environmental contamination and radiation effects.

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1. Transfer of radioactive and associated pollutants in food chains.

a) Transfer in terrestrial ecosystems.

Direct contamination studies of clover and rye plants by spraying were continued under green house and phytotron conditions to ascertain the retention of ^{131}NaI and $^{85}SrCl_2$. The experimental scheme was developed in order to provide information concerning the following points:

- retention at various time intervals after a single spraying (3,24,72,168, 336 and 504 h);
- retention after a single spraying at various rates (4,8,12 and 16 mm/h during one hour);
- retention by the plants after a single spraying and after two successive sprayings at a 24 hour interval;
- retention after a single spraying for various durations (1,2,3 and 4 h);
- retention by the soil and subsequent absorption by the roots or by the shoots after harvesting of the plant;
- biological half-life of the elements in the plant harvested and dried as hay;
- difference of retention of I-131 applied alone or mixed with Sr-85.

The conclusions of this study will be drawn after elaboration of the data.

Studies of the bahaviour of radioiodine (in view of I-129) in soils have been intensified this year.

The distribution of iodine in the system soil-soil solution was measured for a group of seven European soils (Eurosoils), a tropical soil and a clay mineral (illite). The sorption isotherms, determined in soil suspensions with $^{131}I^-$ as a tracer, were linear in all soils up to solution concentrations of iodine of at least 1×10^{-3} ppm. At higher solution concentrations, the isotherms tended versus a saturation curve except for the

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tropical soil where it remained linear up to iodide concentrations of 3.3 ppm. Sorption was completed in all cases in 70 hours, the equilibrium being reached faster at a low pH of the soil.

At the low concentration range of practical interest for radioiodine, the maximum difference in sorption behaviour of the soils were between a loess and a pseudo-gley soil. For a given solution concentration, the loess soil sorbed four times more iodine than the pseudo-gley, while sodium-illite sorbed only one sixth of the loess soil.

In medium term experiments of iodine evaporation from $Na^{131}I$ solutions applied to soil discs exposed in a phytotron, about 4% of the iodine was released in a period of 5-6 days. No further release could be noted after 11 days. The bulk of the evaporation seems to take place the first day after application. In the concentration range from that of carrier free I-131 to 100 ppb I, no concentration effect on the release of iodine could be noted. No vapor loss of iodine could be measured from a loess soil which had reached sorption equilibrium with a $Na^{131}I$ solution prior to evaporation.

The chemical form of iodine evaporated from soil after the addition of a $Na^{125}I$ solution was determined by gas chromatography combined with a thin crystal scintillation detector. Methyl iodide, ethyl iodide and in one case iodate could be shown to be present in air samples above a water-logged podzol, while no molecular iodine was detectable. When a podzol was wet to field capacity with the radioiodine solution, it released measurable amounts only of methyl iodide, with peak concentrations one hour after application. At an iodide concentration of 10 ppb in the wetting solution, the evaporation rate of iodine, all in the form of methyl iodide, was in the first hour at least 5×10^{-7} $\mu g.cm^{-2}.h^{-1}$. The release from a loess soil at field capacity was only about one tenth of that from the podzol and no specific chemical forms could be identified.

As a first part of studies on the uptake of iodine from soil solutions, the rate of uptake of KI by plant root systems at low concentrations (1m μM and 1 μM) was investigated. Excised roots of bean (Phaseolus vulgaris var. Berna), 10-15 days old and grown in 0.2 mM $CaSO_4$-solution were used. In order to measure the actively accumulated iodine, an analysis of the rate of exchange and diffusion of I^- between the cellular compartments-"free space", cytoplasmic phase and vacuole - and washing solution was performed, at 2-4°C. The isotope I-131 was used to label the uptake solution. The most rapid exchangeable phase was rate limiting for less than 20 minutes, followed by the cytoplasmic phase which was rate limiting for 6-7 hours. A remar-

kably good separation of the phases was achieved even at a low 1 mμM I^-. Preliminary results obtained by this method indicated an uptake rate by excised roots of 5.10^{-3} mμ mol I^-/g fr.w. per hour, at a concentration of 1 mμM I^- in the uptake solution.

Studies of the transfer of Zn-65 and Cd to irrigated rice were continued both in controlled conditions (water culture) and in rice fields (experimental station, Vercelli).

The influence of Cd concentration on the absorption, chemical and cellular distribution of Zn-65, was investigated on young rice plants kept in water culture (Hoagland nutrient solution 0.07 ppm Zn) during two months at three Cd concentrations (0 - 0.01 - 0.1 ppm).

- Roots accumulated around 30% of the activity, while the remaining 70% were transferred to the leafy shoots. The main fraction (around 80%) of the root activity can be considered as really absorbed.
- A systematic trend was observed in the chemical distribution of Zn-65 in the roots, in relation to Cd concentration: 57-64 and 47% was bound to the proteinic fraction at 0 - 0.01 and 0.1 ppm Cd respectively.
- The filtration of the root homogenate provided, at the same Cd concentrations, unfiltered fractions amounting to 61, 62 and 70% of the accumulated Zn-65 respectively. The ultra-centrifugation of the filtrate indicated 82-74 and 75% of Zn-65 in the soluble cytoplasm. By chromatographic purification of that soluble fraction, it could be shown that Cd and Zn were associated to two different compounds (a cysteine-peptide of mol. weight 3000-5000 for Cd, a lower mol. weight compound without cystein for Zn).

Studies in a rice field ecosystem included the determination of stable Zn and Cd in water, soil and plants, under various degrees of fertilization. The first results do not indicate any significant effect of the nitrogen fertilizers, as would be expected from their low Zn and Cd contents. The mean concentrations in the various components were the following:

- Irrigation water 38,5 ppb Zn and 1.3 ppb Cd
- Extractable fraction from soil(HCl2N) 45 ppm Zn and 0.36 ppm Cd
- Roots 53 ppm Zn and 2 ppm Cd
- Leafly shoots 42 ppm Zn and 0.67 ppm Cd
- Caryopsis 25 ppm Zn and no detectable Cd

The samples concerned with the eventual effect of phosphorus fertilizers containing relatively high amounts of Zn and Cd, have been collected but not yet analyzed.

Two other research theme on the transfer of cadmium to rice are in progress as part of a joint project with the Chemistry Division of the Joint Research Centre, Ispra. They include the model calculation of the cadmium balance of a rice field on a theoretical basis and a green house experiment on the effect of four levels of soil cadmium on the yield of rice. The analysis of the collected samples is actually in progress.

As far as the effects of radiation on plants are concerned, the effect of high radiation doses on rice has been investigated, taking as a parameter the reflectance in the visible range (400 - 900 nm) of plants issued from rice seeds irradiated with doses from 5 to 30 krad. This range corresponds to the doses which probably reduce the chlorophyll activity. The elaboration of the numerous data accumulated is in progress.

b) Transfer in aquatic ecosystems

Transfer of Zn-65 and Cd-109 to freshwater fish

- In order to check conclusions previously reported, a complementary experiment was conducted with the goldfish, Carassius auratus and Pelmatochromis subocellatus, fed synthetic food containing Cd at concentrations 1000 times higher than that in water (i.e. 0, 20, 40 ppm Cd in food; 0, 20, 40 ppb in water) at the same specific radioactivity. The results confirm 1) the predominance of direct absorption for both radionuclides in Pelmatochromis (75-90%) but not in goldfish (40-55%); 2) the rapid attainment of equilibrium for Cd-109 (after 6-12 days); 3) and accumulation of Cd which is less than proportional to that in water; 4) an inhibition of the Zn exchange with 40 ppb Cd in water; 5) the far greater concentration factor for Zn-65 than for Cd-109.

Distribution of the absorbed radionuclides among the body parts of goldfish indicates the internal organs and the head as sites of major accumulation (75% of total Cd-109 in the internal organs and 36% of total Zn-65 in the head).

- A "natural" food chain including water-organic sediment-benthonic larvae feeding on sediment (chironomids) and fish feeding on larvae (Haplochromis burtoni), was set up in conditions of equilibrium between the various components. The experiment was performed with Lake Maggiore water to which 10 and 20 ppb Cd^{++} was added. Results indicate a high level of accumulation of both radionuclides in the organic sediment (concentration

factor dry sediment/water around 8000 for Cd-109 and 11000 for Zn-65. In chironomid larvae, a strong inhibition of Zn uptake was evident in the presence of Cd in water while the level of Cd-109 accumulation was particularly high (concentration factor larvae w.w./water: 87-167 for Zn-65 and 780-710 for Cd-109, at 20 and 10 ppb Cd in water respectively). In spite of this high level of accumulation in food organisms, the contribution of direct absorption by fish remained predominant (around 90% for Zn-65 and 95% for Cd-109) and the level of accumulation was much lower than in the ingested food organisms (concentration factor fish/water around 130 for Zn-65 and 100 for Cd-109). No inhibition of the Zn exchange by fish in the presence of 20 ppb Cd was evident in this experiment.

- The transfer of both radionuclides was investigated in a two week experiment at different Cd concentrations, along another food chain: mollusk, young Lymnaea stagnalis, 0.5-0.6 mg dry w., to fish, young Haplochromis burtoni, 0.7-0.9gw.w.

Direct accumulation of both radionuclides by the snail was linear with time in absence of stable Cd, the concentration factor (CF snail dry w./water), after 14 days, was around 6800 and 2800 respectively. In the presence of 40 ppb stable Cd, the accumulation of both radionuclides was strongly reduced (CF around 1000 and 1800 respectively), while the stable elements were found at concentrations around 160 ppm Zn and 70 ppm Cd, dry weight.

The transfer of radionuclides to fish living in radioactive water and ingesting snails in equilibrium with water, was found to be a complex function of: the transfer water-snail; the amount of snails ingested by fish; the degree of retention of the ingested radionuclides; and finally the contribution of direct absorption to the total accumulation. Values of the various parameters of this function were determined. They indicate the high contribution of intestinal absorption for Zn-65, which decreases somewhat in the presence of 40 ppb Cd in water (71 to 58%) as a consequence of the strong inhibition of intestinal absorption of radiozinc in the presence of Cd.

2. Chemico-biological interactions.

Methods previously standardized (specific ion electrode, ion exchange resin) have been used with the scope of determining the equilibrium concentrations of metal ions and complex forming species. This experimental study has been mostly concerned with Cd in simple systems (nutrient solutions for plants, isocitrate, NADP, humic acid) under various conditions of pH and ionic strength. The results have been applied to the study of the activation by cadmium of the NADP- dependent isocitrate -dehydrogenase, an enzyme of fundamental importance in cell respiration; it has been shown that the complex cadmium - isocitrate constitutes an excellent substrate for the enzyme.

From experimental results, various calculation procedures have been elaborated for the determination of equilibrium concentrations in more complex systems (natural waters, toxicity test media, incubation media for measurement of enzymatic activity.....).

The biosynthesis of the Cd-binding protein (metallothionein) was investigated in rat liver by simultaneous incorporation of Cd-109 and ^{35}S-cysteine. The results indicate that the synthesis is controlled by the cadmium intake, with a dose proportional increase in both Cd incorporation and Cd-BP synthesis. This increase is linear at single doses of cadmium up to 1 mg Cd^{++}/kg body weight and becomes asymptotic at higher doses, thus suggesting a limit in the capability of the biosynthesis of Cd-BP.

In trout treated with i.p. injection of Cd^{++}/Cd-109, a Cd-BP was found in liver, kidney, spleen and testis. It represents generally the most important binding site of cadmium (up to 90% of the element present in liver, kidney and spleen) while in the testis, cadmium was found equally distributed between the Cd-BP and proteins of high molecular weight.

In tomato plants treated with cadmium, the soluble cytoplasmic fraction of both roots and leaves, contains a major portion of the accumulated cadmium (62 and 69%). A cadmium-binding component could be isolated from that fraction by gel chromatography and was shown to incorporate ^{35}S-cystine, ^{35}S-cysteine.

3. Radioentomology.

Studies on the effects of monoenergetic fast neutrons (4.96 MeV) on Dacus oleae Gmelin and Ceratitis capitata Wiedemann have been developed. They indicate lethal doses of 761, 1521 and 2979 rad for eggs, larvae and pupae

respectively, with a slightly greater resistance of Dacus. A high level of sterility is achieved in adults irradiated at 2979 rad. Fast neutrons appear to be more effective than gamma irradiation in causing mortality in the preimaginal stages as well as dominant lethal mutations in the adult. The use of fast neutrons could therefore be more valid in the "sterile male technique" with those species.

The eventual possibility of using sterile males of Ceratitis capitata in genetical control has been contemplated, through production of chromosomic translocations or pericentric inversions at low irradiation doses. The validity of using substerile males for the genetical control of the two mentionned species has been in any way confirmed.

Laboratory and field investigations on the same two irradiated or non-irradiated species have been carried out on the horizontal and vertical distribution of the pupae in soil as well as on the edaphic factors (chemical composition, structure and moisture of soil) which influence pupation and consequently emergence, propagation of the populations and their fluctuations. Among these factors, soil structure and its moisture play a prominent role. Under each tree crown, a decreasing gradient of the pupae density in soil is observed from South to North. The insects generally pupate in the superficial 5 cm layer, which does not protect them from mortality caused by temperature extremes and by predation.

Bioecological field surveys of Dacus oleae have been continued in Liguria with the capture of adults with chromotropic yellow traps and direct examination of the preimaginal stages in olive and in soil samples. Noticeable phenological differences are evident according to temperature; for ex. at mild temperatures, close to the sea, adults are present all the year round and maximal catches occur one month earlier than in colder hill stations.

A continuous mathematical model has been elaborated on the basis of field observations on Tripetidae, in order to accurately describe the dynamic processes of all the polyvoltine species. Scope of the model was to estimate parameters which are difficult to observe like fecundity and mortality.

Radiosensitivity of the various life stages of two insects harmful to the nut-tree (Gonocerus acuteangulatus Goeze, Rhyncota and Altica brevicollis Foudras, Coleoptera) has been investigated. Along this line, preliminary results have been obtained on various species harmful to the chestnut (Cydia splendana Hb., Cydia amplana Hb., Lepidoptera, and Balaninus elephas Gyll., Coleoptera).

A parameter useful to establish a radiosensitivity scale among insects (radiation dose which decreases by 50% the survival time ST_{50} of a natural population) has been checked successfully on six noxious species of Coleoptera (Stegobium paniceum L., Rhizopertha dominica F., Orizaephilus surinamensis L., Cryptolestes turcicus Grouv., Sitophilus orizae L., Sitophilus granarius L.).

Finally a study has been made on the consumption of wheat grain by adults of Sitophilus granarius L., in relation to the irradiation of the grains. The consumption by successive generations seems to decrease when irradiated grain is consumed. Further study is necessary to establish the causes of this phenomenon.

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Results of Project No. 2

Head of Project and scientific staff: F.Campagnari, L.Clerici, M.Talpaert

Title of Project: Genetical Biochemistry. DNA damage by radiation and mutagenic chemicals. Mammalian mechanisms involved in the enzymatic expression and the repair of this damage.

- Preparation of polydeoxynucleotides

The activity in this field was continued within the frame of references and according to the criteria established in the preceding years. Radioactive and non-radioactive polydeoxynucleotides of the dT, dA and the dC series were prepared and assembled in macromolecular complexes mimicking specific substrates and templates for the enzymatic repair and replication of DNA.

Polydeoxynucleotide-oligodeoxynucleotide pairs of a template-initiator structure with pyrimidine dimers and radioactively labeled bases in the template strand were produced. These systems were successfully used to investigate whether DNA polymerases promoted correct or incorrect catalysis when radiation damage was present in the template chain directing the formation of new DNA.

A number of dT nucleotides of increasing molecular size were prepared and isolated to analytical purity in relative large quantities (up to 100 mg for each member of the series). The oligomers with chain number up to 6, namely $(pdT)_2$, $(pdT)_3$, $H_2N\text{-}dT\text{-}(pdT)_5$ and $(pdT)_6$, were chemically synthesized in a stepwise manner according to the phosphotriester method of oligonucleotide synthesis. The high-molecular weight $(pdT)_{30}$, $(pdT)_{100}$ and $(pdT)_{200}$ were obtained by enzymatic elongation of oligodT under controlled conditions. The chemical synthesis of the oligothymidylates was part of a collaboration with the Gorlaeus Laboratoria of the University of Leiden.

An oligodT-cellulose enriched in the nucleotide moiety and with a stable peptide bond between the 5' end of the dT chain and the hexose units of the polysaccharide matrix was obtained by reacting $H_2H\text{-}dT(pdT)_5$ with cyanogen bromide - activated cellulose. This is a new approach for coupling dT sequences to insoluble particles, that is, for the initial limiting step in the preparation of polydeoxynucleotide-cellulose complexes.

The old and new types of polydeoxynucleotides were largely distributed to outside laboratories engaged in researches on DNA repair within the present pluriannual Programme.

- Analytical methods for studying normal and irradiated DNA

An ESR spectrometer combined with a van de Graaff accelerator was transformed as to accept liquid samples. The change of the detection-irradiation chamber in a flow-cell system allowed to reveal radicals formed during irradiation of nucleotides in aqueous solutions. Radicals of pyrimidine bases were identified and the overall ESR data had distinctive features with respect to the results obtained on nucleic acid material irradiated in the solid state.

The conformation of DNA-like molecules in aqueous media was investigated with the aid of high field NMR spectroscopy. Using the series of dT nucleotides with increasing polymerization number, detailed assignments of proton spectra were achieved. Chemically equivalent protons could be differentiated with respect to their location at the 5'-end, 3'-end and the interior of dT chains. NMR pattern of proton in irradiated samples gave direct information on the molecular nature of the radiation damage.

Optical spectroscopic procedures for studying the interaction between polarized light and matter were adjusted as to analyze DNA solutions subjected to alternating electric fields of variable frequence. The experimental system allowed to align the DNA molecules in solution and to detect differential refraction and differential absorption of the incident polarized light as due to the induced anisotropy of the oriented DNA helices. The observed data on the refraction (birifringence) and the absorption (linear dichroism) of the incident light gave direct indication of the structure of normal and irradiated DNA molecules in aqueous media.

Biochemical methods provided circumstantial evidence that the 3' end of DNA breaks caused by X-irradiation may carry OH groups which do not react with the specific enzymes used to label 3'-OH termini in non-irradiated DNA.

- Enzymes of DNA repair and DNA replication

Mammalian DNA polymerases were found able to bind proficiently on 3' termini on initiator - template DNA structures which have been heavily irradiated and apparently did not contain 3'-OH terminal functions. These enzymes were also shown to be adversely affected in an unspecific manner

by antibiotics, such as lipiarmycin and rifamycin dimers, which are active on bacterial DNA polymerases.The inhibition of enzyme activity by these compounds was not correlated with their lipophilic properties.

The distribution pattern of DNA polymerases in nuclear extracts of calf thymus cells showed that a major component of the recovered enzymatic activity was a DNA polymerizing system of high molecular weight. This enzymatic system was apparently heterogeneous and unstable. Incorrect conditions in the preparation and storage of the nuclear extracts led to loss of the enzyme activity with the persistence of the catalysis of the minor DNA polymerase with a sedimentation coefficient of 3.4 S.

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Results of Project No. 3

Head of Project and scientific staff: J.Booz, M.Coppola

Title of Project: Radiation structure of ionizing radiations in tissue and it relation to their spectral energy deposition in biological structures and to the biological effectiveness.

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a) Radiation structure in biological material and in model substances.

The radiation energy of directly and indirectly ionizing radiations is transferred to matter via secondary electrons. Most of these electrons have an energy of less than 1 keV. Measurement of the W-value of such low energy electrons are therefore not only significant for the calibration of ionization chambers, but also relevant for obtaining basic information on the concentration of ions in biological tissue. The experiments on the W-value of electrons of 20 eV to 5 keV for various gases and gas mixtures were continued. The measurements were concentrated on the problem of the so far unexplained peak in the W-value between 200 and 1000 eV and on the improvement of experimental conditions.

The relation between the differential W-value and the integral W-value and the integral W-value of charged particles, as well as the effective W-values of indirectly ionizing radiations was analyzed theoretically. It was shown e.g. that the effective W-value of fast neutrons can not be obtained by averaging over the energy spectrum of the recoil ions. With fast neutrons the size of the sensitive gas volume cannot be neglected and therefore the problem of the effective W-value has to be solved with the help of cavity theory.

The evaluation of experimental results on LET, the distance restricted linear energy transfer, of protons and deutrons was continued. Informations on the fluctuation of LET, for radical distances between 100 Å and 0.15 µm were also obtained.

b) Evaluation of the energy deposition of different types of radiations to small biological volumes and its relation to the corresponding biological effectiveness.

Energy deposition spectra of X-rays, gamma-rays, and fast neutrons give information on the radiation structure of these radiations in biological tissue. In addition the mean energy deposition in simulated radiosensitive biological volumes are characteristic measures of radiation quality. Hence both data are relevant for the understanding of radiation mechanism.

The experimental studies on the spectral energy deposition of low LET-radiations were continued using mainly the cylindrical walled and wall-less counters. It is the scope of these measurement to obtain new information on the wall effect. An analysis of our data and of the data given in the literature showed that there exists a unique relation between the mean lineal energie $\bar{y}_F$ and $\bar{y}_D$ and the volume diameter d. This relation is the same for all analyzed low LET-radiations, but varies with the counter shape and is also slightly different for walled and wall-less counters. Fig. 1 shows as an example the relation between $\bar{y}_F$, $\bar{y}_D$, and d for cylindrical walled counters. The straight line in this figure permits the evaluation of $\bar{y}_D$ if $\bar{y}_F$ and d are known.

Experimental energy deposition spectra of fast neutrons are often perturbed by the presence of collimation as well as of materials surrounding the exposure area. For this reason a study was carried out to view the influence of the collimation on the shape of the energy deposition spectra of fast neutrons in a simulated biological volume of microscopic dimensions. The results show that in general the presence of a collimation increases the low LET contribution to the energy deposition spectrum. Conversely, in case of no collimation the presence of degraded neutrons resulting from scattering in the walls and floor of the irradiation room can be as relevant as to mask the effect of the direct neutrons. As a consequence the deposited energy spectrum shape becomes much less sensitive to variations of the primary energy.

The RBE of fast neutrons for the production of certain biological effect is known to depend on neutron energy as well as on dose or dose rate. Experiments aiming to give such quantitative informations at low doses are, however, generally difficult for living animals. The opacity formation in the eyelenses of mice due to neutron interaction appears as a promising

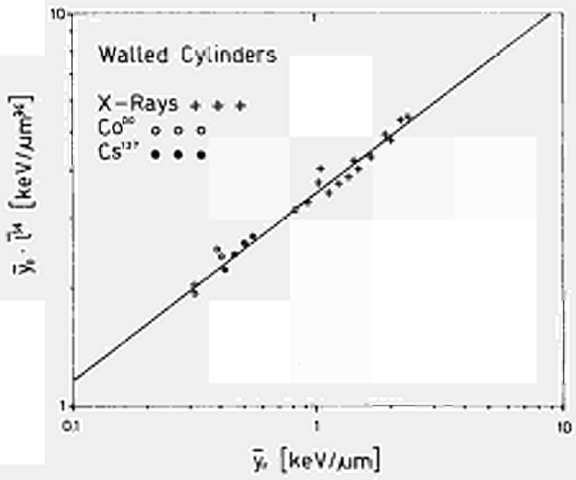

Fig. 1 : $\bar{y}_D.d^{1/4}$ as a function of y_P for X-and gamma-rays between 12 keV and about 1.6 MeV. The data were measured with wall-less spherical proportional counters.

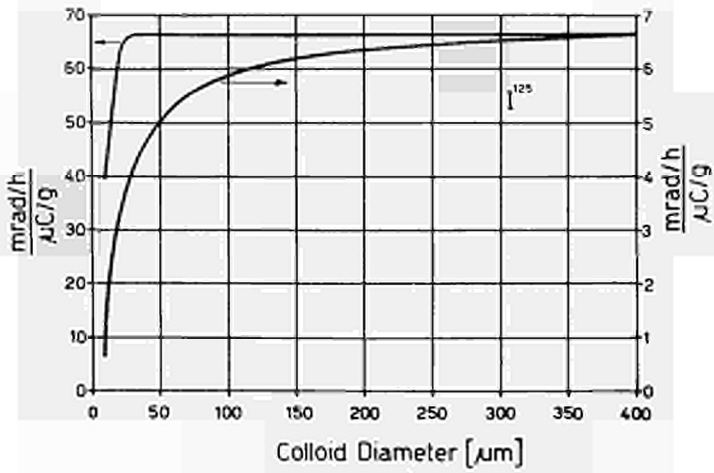

Fig. 2 : Electron dose rate per µC/gram thyroid at the center (left ordinate) and at the nuclei of the follicle cells (sight ordinate) as a function of the diameter of the follicle colloid.

effect to be studied in this respect. Therefore an experimental study on this subject was undertaken in collaboration with a group of CNEN/Casaccia. A first set of 300 mice was irradiated with neutrons from 0.4 to 5 MeV and total doses from 1 to 40 rad. The first results will be available next summer.

The problem of radiation dosimetry in a mixed field of neutrons and gamma-rays has been extensively investigated in the framework of the European neutron dosimetry intercomparison project ENDIP. Four ionization chambers with different sensitivities to neutrons and gammas were brought into operation. Measurements were carried out in various experimental situations appropriate for the experimental determination of the various parameters interveening in the evaluation of doses. Subsequently a complete set of irradiations of dosemeters were performed at GSF/Neuherberg under well controlled beam conditions to be intercompared with the results of similar experiments of the groups. Problems of experimental nature related to the behaviour of the dosemeters, as well as that of the adequacy of the presently available physical data utilized for neutron and gamma-ray dosimetry are still under study.

Experiments on neutron spectrometry using a ^{3}He-spectrometer were continued.

The calculations of the local distribution of the electron gamma dose rate inside and outside of the follicles of a ^{125}I contaminated human thyroid gland were terminated. Fig. 2 shows the electron dose rate at the follicle center and at the position of the nuclei of the cells forming the monocellular layer of the follicles. The total dose rate at this position (32mrad/h per μC/gram thyroid) is nearly independent of the size of the follicles and their relative geometrical position.

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