COMMISSION OF THE EUROPEAN COMMUNITIES

Directorate-General
for Science, Research and Development

Directorate
Biology, Radiation Protection and Medical Research

Directorate-General
Employment and Social Affairs

Directorate
Health and Safety

1980-1984
Radiation Protection Research Programme

CATALOGUE OF CONTRACTS

Volume II

DESCRIPTION OF RESEARCH PROGRAMMES

February 1982

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KATALOG OVER KONTRAKTER
Forskningsprogrammet "Strålingsbeskyttelse"

FÖRDERUNGSKATALOG
Forschungsprogramm „Strahlenschutz"

CATALOGUE OF CONTRACTS
Research programme "Radiation Protection"

CATALOGUE DES CONTRATS
Programme de recherche "Radioprotection"

ΚΑΤΑΛΟΓΟΣ ΤΩΝ ΣΥΜΒΑΣΕΩΝ
Πρόγραμμα ἔρευνας «Προστασία ἀπό τίς ἀκτινοβολίες»

CATALOGO DEI CONTRATTI
Programma di ricerca "Radioprotezione"

CATALOGUS VAN CONTRACTEN
Onderzoeksprogramma „Stralingsbescherming"

1980-1984

Volume II

First Edition

February 1982

CII/466/82-II

EUR 91

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

Forord

Kommissionen har tidligere offentliggjort to udgaver af sit "Katalog over kontrakter" vedrørende dens program "Strålingsbeskyttelse". Formålet med disse publikationer var at give et tydeligere indtryk af Kommissionens program og at virke som en hjælp til dets forvaltning. Publikationerne indeholdt oplysninger om forskningskontrakterne, såsom navne på kontrahenter, ledere af forskningsgrupper, løbetid, budget m.v., som har vist sig at være nyttige for kontrahenterne samt for nationale og internationale myndigheder. De tidligere publikationer indeholdt ikke detaljerede videnskabelige oplysninger om hver enkelt forskningskontrakt, men det er blevet foreslået at medtage sådanne oplysninger i en ny udgave.

Det er derfor blevet besluttet at offentliggøre det nye katalog i to særskilte bind, idet det første bind indeholder administrative oplysninger, mens det andet omfatter en fuldstændig beskrivelse af den forskning, der skal gennemføres under hver enkelt kontrakt. Alle forskningskontrakter, der blev godkendt inden udgangen af december 1981, er medtaget. Senere indgåede forskningskontrakter vil blive omhandlet i kommende udgaver af kataloget.

Af hensyn til læserne er kontrakterne blevet opdelt i seks større grupper, nemlig, strålingsdosimetri og dens betydning, optræden af og kontrol med radionuklider i miljøet, kortsigtede somatiske virkninger af ioniserende stråling, sene somatiske virkninger af ioniserende stråling, genetiske virkninger af ioniserende stråling samt vurdering af strålingsrisikoen. Omfatter en forskningskontrakt flere projekter, der henhører under forskellige grupper, er den klassificeret efter dens hovedforskningsområde. Der er endvidere vedføjet skemaer med visse programforvaltningsdata i tillægget til første bind.

Det er vort håb, at dette katalog vil være til nytte for såvel administratorer som videnskabsmænd. Det bør dog understreges, at der desuden vil blive offentliggjort detaljerede videnskabelige oplysninger i de årlige rapporter om programmet "Strålingsbeskyttelse", og specielle emner behandles i monografier og meddelelser om forskningen i strålingsbeskyttelse.

F. VAN HOECK A.E. BENNETT

Vorwort

Die Kommission hat bereits früher zwei Ausgaben des "Förderungskatalogs" ihres Strahlenschutzprogramms veröffentlicht. Das Ziel dieser Kataloge war es, ein übersichtliches Bild des Kommissionsprogramms aufzustellen und eine Hilfe für die Programmverwaltung zu sein. Sie enthielten Informationen zu den Forschungsverträgen, zum Beispiel Namen der Vertragspartner, Leiter der Forschungsgruppen, Vertragsdauer, Budgetangaben usw. Alle diese Informationen erwiesen sich als nützlich sowohl für die Vertragspartner, als auch für nationale und internationale Behörden. Einzelheiten über die wissenschaftlichen Aufgaben der Forschungsverträge waren in den früheren Ausgaben nicht enthalten. Es wurde jedoch vorgeschlagen, diese in einer neuen Ausgabe aufzunehmen.

So wird jetzt der neue vorliegende Katalog in zwei Bänden veröffentlicht. Der erste Band umfasst die verwaltungstechnischen Informationen, der zweite enthält ausführliche Beschreibungen der in den jeweiligen Verträgen vorgesehenen Forschungsarbeiten. Alle Verträge des Programms Strahlenschutz, die vor Ende Dezember 1981 abgeschlossen wurden, sind aufgeführt. Nach diesem Datum abgeschlossene Verträge werden in zukünftigen Ausgaben berücksichtigt.

Aus Zweckmässigkeitsgründen wurden die Verträge sechs Hauptgebieten zugeordnet, nämlich Strahlendosimetrie und ihre Interpretation, Verhalten und Kontrolle von Radionukliden in der Umwelt, somatische Sofortwirkungen, somatische Spätwirkungen sowie genetische Wirkungen ionisierender Strahlung und Abschätzung des Strahlenrisikos. Wenn Forschungsverträge mehrere Projekte enthalten, die zu verschiedenen Gebieten gehören, so wurden sie entsprechend ihrem Hauptforschungsgebiet eingeordnet. Tabellen mit Angaben zur Programmdurchführung befinden sich im Anhang zum ersten Band.

Wir hoffen, dass dieser Katalog sowohl für den Wissenschaftler als auch für den Verwaltungsbeamten von Nutzen sein wird. Es soll noch darauf hingewiesen werden, dass detaillierte wissenschaftliche Angaben aus dem Strahlenschutzprogramm in den jährlichen Tätigkeitsberichten und spezielle Themen in Monographien und Tagungsberichten veröffentlicht werden.

F. VAN HOECK

A.E. BENNETT

Préface

La Commission a déjà publié deux éditions du "Catalogue des contrats" de son programme "Radioprotection". Ces publications visaient à donner une idée plus claire du programme de la Commission et à faciliter sa gestion. Elles comprenaient des informations sur les contrats de recherche, telles que les noms des contractants, des responsables des équipes de recherche, la durée des contrats, leur budget etc. qui se sont toutes avérées utiles aux contractants et aux autorités nationales et internationales. Il y manquait cependant une description scientifique détaillée de chaque contrat de recherche, et c'est pourquoi il a été suggéré de la faire figurer dans une nouvelle édition.

La Commission a donc décidé de publier le nouveau catalogue en deux volumes séparés, le premier comprenant les informations administratives et le deuxième donnant une description complète des recherches à effectuer dans le cadre de chaque contrat. Tous les contrats de recherche acceptés avant la fin du mois de décembre 1981 y ont été inclus. Les contrats de recherche conclus depuis figureront dans les prochaines éditions du Catalogue.

Pour la commodité du lecteur, les contrats ont été répartis en six grandes catégories, à savoir la dosimétrie et son interprétation, le comportement et le contrôle des radionucléides dans l'environnement, les effets somatiques à court terme des rayonnements ionisants, les effets somatiques tardifs des rayonnements ionisants, les effets génétiques des rayonnements ionisants et l'évaluation des risques dûs à l'irradiation. Si un contrat de recherche est composé de plusieurs projets faisant partie de catégories différentes, il est classé d'après son principal domaine de recherche. De plus, des tableaux comprenant certaines données sur la gestion des programmes figurent à l'annexe du premier volume.

Il faut espérer que ce catalogue s'avérera utile à la fois aux administrateurs et aux scientifiques. Il convient toutefois de souligner que des informations scientifiques détaillées seront également publiées dans les rapports annuels du programme "Radioprotection" et que des thèmes particuliers seront traités dans les séries de monographies et de comptes rendus portant sur la recherche en matière de radioprotection.

F. VAN HOECK A.E. BENNETT

Preface

The Commission has previously published two editions of a "Catalogue of Contracts" of its Radiation Protection Programme. The aim of the publications was to convey a clear impression of the Commission's programme and to serve as an aid for its management. These publications contained information on the research contracts, such as names of contractors, heads of research teams, duration, budget, etc. all of which proved to be useful for the contractors, and for national and international authorities. Detailed scientific information on each of the research contracts was not given in the previous publications, however it has been suggested that this be included in a new edition.

It has therefore been decided to publish the new Catalogue in two separate volumes, the first volume giving the administrative information, the second one giving a full description of the research to be carried out during each contract. All research contracts accepted before the end of December 1981 have been included. More recently concluded research contracts will be included in forthcoming editions of the Catalogue.

For the convenience of the reader, the contracts have been assigned to six major sectors, i.e. radiation dosimetry and its interpretation, behaviour and control of radionuclides in the environment, short-term somatic effects of ionizing radiation, late somatic effects of ionizing radiation, genetic effects of ionizing radiation, and evaluation of radiation risks. Whenever a research contract is composed of several projects belonging to different sections, it is categorized according to its main research area. Furthermore, tables with some programme management data are given in the appendix to the first volume.

It is to be hoped that this Catalogue will prove useful for both administrators and scientists. However, it should be emphasized that in addition, detailed scientific information will also be published in the Annual Reports of the Radiation Protection Programme, and special topics will be dealt with in the Radiation Protection Research monographs and proceedings series.

F. VAN HOECK A.E. BENNETT

Εἰσαγωγή

Ἡ Ἐπιτροπή ἔχει δημοσιεύσει στό παρελθόν δύο ἐκδόσεις "καταλόγου Συμβολαίων" τοῦ Προγράμματος τῆς Προστασίας ἀπό Ἀκτινοβολίες. Ὁ σκοπός τῶν ἐκδόσεων αὐτῶν ἦταν νά δώσουν μία σαφέστερη ἐντύπωση τοῦ Προγράμματος τῆς Ἐπιτροπῆς καί νά χρησιμεύσουν σάν βοήθημα γιά τή διαχείρισή του. Οἱ ἐκδόσεις αὐτές περιεῖχαν πληροφορίες γιά τά ἐρευνητικά συμβόλαια, ὅπως ἐπωνυμίες τῶν ἐργοληπτῶν, τούς προϊσταμένους τῶν ἐρευνητικῶν ὁμάδων, διάρκεια, προϋπολογισμό κλπ. Οἱ πληροφορίες αὐτές ὑπῆρξαν χρήσιμες γιά τούς ἐργολῆπτες καί γιά τίς ἐθνικές καί διεθνεῖς ἀρχές. Στίς προηγούμενες ἐκδόσεις δέν εἶχαν δοθεῖ λεπτομερεῖς ἐπιστημονικές πληροφορίες γιά τό κάθε ἕνα ἀπό τά ἐρευνητικά συμβόλαια, προτείνεται ὅμως νά περιληφθοῦν σέ μία καινούργια ἔκδοση.

Ὡς ἐκ τούτου, ἀπεφασίσθη ὁ καινούργιος κατάλογος νά δημοσιευτεῖ σέ δύο χωριστούς τόμους, ἐκ τῶν ὁποίων ὁ πρῶτος θά δίνει τίς πληροφορίες διοικητικῆς φύσεως, καί ὁ δεύτερος πλήρη περιγραφή τῆς ἔρευνας πού θά ἐκπονεῖται μέ κάθε συμβόλαιο. Ὅλα τά ἐρευνητικά συμβόλαια πού εἶχαν γίνει ἀποδεκτά μέχρι τέλους τοῦ 1981 ἔχουν συμπεριληφθεῖ. Τά ἐρευνητικά συμβόλαια πού ἔχουν συναφθεῖ πιό πρόσφατα θά περιληφθοῦν σέ προσεχεῖς ἐκδόσεις τοῦ καταλόγου.

Γιά τή διευκόλυνση τοῦ ἀναγνώστη τά συμβόλαια ἔχουν χωρισθεῖ σέ ἕξι εὐρεῖς τομεῖς, δηλαδή δοσομετρία ἀκτινοβολιῶν καί ἑρμηνεία της, συμπεριφορά καί ἔλεγχος τῶν ραδιονουκλεϊδιων στό περιβάλλον, βραχυχρόνιες σημαντικές ἐπιδράσεις ἀπό ἰονίζουσες ἀκτινοβολίες, μακροπρόθεσμες σωματικές ἐπιπτώσεις ἀπό ἰονίζουσες ἀκτινοβολίες καί ἀξιολόγηση τῶν κινδύνων ἀπό τίς ἀκτινοβολίες. Σέ ὅσες περιπτώσεις ἕνα ἐρευνητικό συμβόλαιο ἀποτελεῖται ἀπό περισσότερα ἀπό ἕνα προγράμματα πού ἀνήκουν σέ διαφορετικούς τομεῖς τότε αὐτό ταξινομεῖται σύμφωνα μέ τό ἀντικείμενο τοῦ βασικοῦ του ἐρευνητικοῦ τομέα. Ἐπίσης, πίνακες μέ ὁρισμένα στοιχεῖα διαχειρίσεως τοῦ προγράμματος δίνονται στά παραρτήματα τοῦ πρώτου τόμου.

Ἐλπίζεται ὅτι αὐτός ὁ Κατάλογος θά φανεῖ χρήσιμος τόσο σέ διοικητικούς ὑπαλλήλους ὅσο καί σέ ἐπιστήμονες. Πρέπει ὅμως νά τονισθεῖ ὅτι λεπτομερεῖς ἐπιστημονικές πληροφορίες θά δημοσιευθοῦν ἐπίσης στίς ἐτήσιες ἐκθέσεις τοῦ Προγράμματος Προστασίας ἀπό Ἀκτινοβολίες, καί τά εἰδικά θέματα θά ἀντιμετωπισθοῦν στίς σειρές Μονογραφιῶν καί Πρακτικῶν τῆς Ἔρευνας γιά Προστασία ἀπό Ἀκτινοβολίες.

F. VAN HOECK

A.E. BENNETT

Prefazione

La Commissione ha pubblicato in passato due edizioni di un "Catalogo dei contratti" conclusi nel quadro del Programma di Radioprotezione, nello scopo di meglio illustrare tale programma e di renderne più agevole la gestione. Le informazioni riguardanti i contratti di ricerca, come per esempio i nomi dei contraenti e dei responsabili dei gruppi di ricerca, la durata dei progetti, il bilancio previsto, ecc. si sono rivelate utili sia per i contraenti che per le autorità nazionali ed internazionali. Di nessuno dei contratti di ricerca si comunicavano dati scientifici, ma è stato suggerito di includere questo aspetto in una nuova edizione.

Si è pertanto deciso di pubblicare il nuovo catalogo in due volumi distinti, il primo contenente informazioni amministrative, il secondo una descrizione completa delle attività di ricerca previste in ogni contratto. Nel catalogo figurano tutti i contratti di ricerca conclusi prima della fine del 1981. I contratti più recenti figureranno invece in future edizioni del catalogo.

Per comodità di lettura i contratti sono stati ripartiti in sei settori principali: dosimetria delle radiazioni e sua interpretazione, comportamento e controllo dei radionuclidi presenti nell'ambiente, effetti somatici a breve termine delle radiazioni ionizzanti, effetti somatici tardivi delle radiazioni ionizzanti, effetti genetici delle radiazioni ionizzanti, valutazione dei rischi da radiazione. I contratti articolati in più progetti, relativi a settori diversi, sono repertoriati nel settore corrispondente al progetto più importante. Infine, tabelle contenenti dati di gestione del programma sono contenute nell'appendice allegata al primo volume.

Si spera che questo catalogo si riveli utile sia per i responsabili amministrativi che per i ricercatori impegnati nei vari contratti. Desideriamo comunque far presente che altre informazioni scientifiche dettagliate saranno pubblicate nelle relazioni annuali del Programma di Radioprotezione e che argomenti particolari faranno oggetto di monografie e di rapporti dedicati alla ricerca nel campo della Radioprotezione.

F. VAN HOECK A.E. BENNETT

Voorwoord

De Commissie publiceerde reeds eerder twee uitgaven van een "Catalogus van Contracten" van haar onderzoeksprogramma Stralingsbescherming. Met de publikatie ervan werd beoogd een beter inzicht te geven in het programma van de Commissie en een hulpmiddel te vormen bij het beheer van dat programma. De publikaties in kwestie bevatten informatie over de onderzoekscontracten, zoals naam van de contractanten, leiders van de onderzoeksgroepen, duur, uitgetrokken kredieten, enz. Al die gegevens zijn nuttig gebleken voor de contractanten, alsmede voor de nationale en internationale instanties. Een gedetailleerde wetenschappelijke informatie met betrekking tot ieder van de vermelde onderzoekscontracten was in de vroegere publikaties evenwel niet opgenomen; wel werd voorgesteld dit soort informatie in een nieuwe uitgave te verstrekken.

Er werd derhalve besloten de nieuwe catalogus in twee afzonderlijke delen te publiceren, namelijk een deel met de administratieve informatie en een ander met een volledige beschrijving van de onderzoekswerkzaamheden die in het kader van ieder contract moeten worden uitgevoerd. Alle voor het einde van december 1981 goedgekeurde onderzoekscontracten zijn in de lijst opgenomen. De contracten die op latere data werden afgesloten, zullen in de komende edities van de catalogus worden opgenomen.

Ten gerieve van de lezer zijn de contracten in zes hoofdsecties ingedeeld, namelijk stralingsdosimetrie en de interpretatie hiervan, gedrag van en controle op radionucliden in het milieu, vroege somatische effecten van ioniserende straling, late somatische effecten van ioniserende straling, genetische effecten van ioniserende straling en beoordeling van stralingsrisico's. Telkens wanneer een onderzoekscontract projecten omvat die tot verschillende secties behoren, is dit volgens het belangrijkste onderzoeksgebied ingedeeld. Bovendien zijn in het eerste deel tabellen opgenomen met bepaalde gegevens inzake het programmabeheer.

Deze catalogus zal hopelijk van nut zijn zowel voor administrateurs als voor wetenschappers. Gedetailleerde wetenschappelijke informatie zal voorts nog worden gepubliceerd in de jaarverslagen van het programma Stralingsbescherming, terwijl speciale onderwerpen zullen worden behandeld in de monografieën en rapporten betreffende het onderzoek op het gebied van de stralingsbescherming.

F. VAN HOECK A.E. BENNETT

INTRODUCTION

THE RADIATION PROTECTION RESEARCH PROGRAMME OF THE COMMISSION OF THE EUROPEAN COMMUNITIES

Research on radiation protection was one of the subjects originally mentioned in the Treaty of Rome. Since 1959, when the first research contracts with national institutions were signed, the Commission has continued and developed this Radiation Protection Programme. Considering the progress of knowledge, but also the changing requirements for protection in the Community, an important evolution in the scientific content of the programme has taken place. A new 5 year research programme 1980-1984 is currently being carried out.

Recent events have indicated the pertinence of radiation protection. In any accidental situation the first problems to be solved concern the safety of the population. The first questions to be answered concern the risk for the population, the detriment, the late effects to be anticipated and the prevention, control and treatment of potential radiation damage to man and his environment.

The role of this scientific research programme is to supply sufficient information to contribute to a framework so that informed decisions can be made on issues to which public opinion has become very sensitive. Therefore, the Radiation Protection Programme of the Commission is designed to gain a satisfactory understanding and control of radiation risks with two main objectives:

- improvement of scientific and technical knowledge with a view to updating basic standards for the health protection of the general public and workers against the hazards arising from ionizing radiation,
- evaluation of the biological and ecological consequences of nuclear activities and of the use of nuclear energy and ionizing radiation, in order to ensure an adequate protection of man and of the environment whenever unacceptable harm could otherwise be caused to them.

It is evident that these objectives directly concern Community policies of undisputed importance: the social, the environment and the energy policies. To reach these objectives, a co-operative European effort is necessary on a broad variety of subjects forming a closely integrated programme in radiation protection research including measurement of radiation dose, environmental pathways, degree and control of environmental radiocontamination, prevention and treatment of short-term and late somatic effects and their consequences, the role of genetic effects for future generations and the assessment of consequences of radiation for man and his environment.

The scope of the subjects ranges from very pertinent problems, such as the effects of low level irradiation, technologically enhanced levels of exposure from natural radioactivity, indoor exposure, and comparative risk assessment, to others becoming more important in the future, when fusion energy might be available, such as the radiotoxicity of tritium. Studies on the biological consequences of handling of waste, effluent and fuels throughout their cycles are important for decisions on safe energy provision, while others aim at reducing the most important source of man-made irradiation of the whole population by improving radiation techniques used in medical diagnosis.

In 1981, more than 900 scientists in the Community worked full- or part-time within more than 190 contracts including some 310 research projects, to achieve these vital goals.

This effort, expressed in man-years for the total duration of the contracts, amounts to up to more than 1100 man-years of scientific input and over 1000 man-years of input by other collaborators. The total cost of the planned research adds up to about 103 MioECU with a financial participation of the Commission of the European Communities of over 37 MioECU.

The collection of research contracts included in this edition of the "Catalogue of Contracts" reflects the situation as of 31 December 1981. Meanwhile new proposals have been submitted to the Commission and they have been discussed by the Advisory Committee on Programme Management. They will be included in a forthcoming updated edition.

I. LIST OF CONTRACTS

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
Prof. G.W. Barendsen
Radiobiological Institute
TNO
P.O.Box 5815
NL-2280 HV Rijswijk

Contract no.: BIO-A-300-81-NL

General subject of the contract:

Evaluation of the biological effectiveness of various types of radiation for different types of damages in cultured mammalian cells.

Description of research work:

The aim of the research programme is to obtain quantitative information and insights in mechanisms, concerning various cellular responses to radiation, necessary to elucidate the processes that lead to somatic effects in mammals, especially tumour induction.

The experiments to be carried out will pertain to three different cellular responses, namely cell reproductive death, chromosome aberrations and malignant transformations, because these are all of importance in determining the probability of cancer development in animals.

Studies on the effectiveness of different types of radiations for these cellular effects will be carried cut with cultured cells of different origin because radiation quality influences the shapes of dose-response curves and the differences observed can provide insights in mechanisms. Radiations of high linear energy transfer (LET) or lineal energy (y) generally result in dose-effect relationships which are less difficult to analyse and interpret, because accumulation of damage and repair of damage are of lesser importance as compared to low LET radiations.

Experiments to compare responses of different types of cells will be carried out because, although the underlying mechanisms of radiation action may be similar, quantitative differences are expected to be of great importance and this information may help to elucidate the influence of various factors. Investigations of the biological effectiveness of fast neutrons of different energies will be made for induction of the effects mentioned, because the dependence on neutron energy is not only important with respect to radiation protection, but also with respect to the analysis of mechanisms.

Investigations with single and fractioned exposures will be carried out in order to analyse the influence of repair processes. The information and insights obtained with respect to cellular responses will be employed to interpret data on tumour induction which will be derived from other programs. In view of the large numbers of animals and the long observation times involved in studies on carcinogenesis in animals, is it impossible to investigate experimentally the influence of all relevant factors and parameters. Therefore information for less complex systems, such as cells in culture, is necessary to provide a fundamental basis for an optimal interpretation of the less abundant animal data, which require a large amount of work and facilities.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
Prof. D. Blanc
Centre de Physique Atomique
Université Paul Sabatier
Route de Narbonne 118
F-31077 Toulouse Cédex

Contract no.: BIO-A-295-81-F

General subject of the contract:

Transport simulation of particles, and measurement of the ionization potential of some polar liquids approximating to biological media.

Description of research work:

1. Particle transport

The general aim of the first point of the research programme, is to obtain, by means of numerical simulation, as much useful information as possible about (a) the elementary energy deposits brought about by radiation in matter, and (b) the various events induced when energy is deposited. It will be possible to obtain statistics on the quantities which are significant in relation to the action of radiation and, in particular, the various microdosimetric functions, spatial distribution of ionization and of inter-ionization distances, LET distribution, average LET, etc. Transport of electrons will be considered in the case of systems simulating cells, cellular organelles and systems constituted by DNA molecules and those related to them. There is still much to be done in this field to take account of interaction of electrons with the molecules of living tissues and to improve knowledge of their effectiveness in the various primary radiobiological processes.

The simulation of photon transport will be improved at low energy levels (below keV level) by research and use of effective cross sections better adapted to this energy field and to the irradiated materials, thereby extending the previous study. The transport of protons, heavier ions and neutrons will be simulated with a view to solving problems in connection with the dosimetry of particles with high LET. An attempt will be made to interpret and iron out existing divergences by means of analysis and accurate simulation of trajectories and events, which will also make it possible to simulate ion traces, and the generation and deceleration of delta rays. Using the set of techniques that have been developed, it will be possible to consider all secondary particles, up to an energy level at which they can no longer ionize regardless of how they are generated and whatever the nature of the particles of the incident beam.

The case of mixed neutron-gamma fields will also be dealt with; applications will be concerned with their calculated dosimetry, the theoretical response of the detectors used and calculation of response functions.

2. Ionization potential measurements

Former work of this laboratory proved that the ionization potential values of non polar liquids (mainly hydrocarbons) are lowered by about 15 % in going from the gaseous state to the liquid state. Such a

lowering was expected but had not yet been exactly measured. After modification of the experimental set-up built for the above-mentioned studies, the contractant proposes to study the influence of the physical target state on the ionization-potential value for higher dielectric constant compounds such as alcools or water. The main aim being to measure the ionization-potential value of the most interesting liquid from a biological point of view namely water.

The ionization potential values of these liquids will be determined by measuring the photocurrent as a function of photon wavelenth between 110 nm and 200 nm. These U.V. photons would be obtained from a microwave-powered lamp filled with a 10 % hydrogen-argon mixture or, if the photon intensity is not sufficient, from an accelerator or a storage ring (synchroton radiation).

From the ionization efficiency curve of each liquid, the law of variation of the ionization quantum yield with photon energy will be deduced.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. D. Blanc
Univ. P. Sabatier
Centre de Physique Atomique
Route de Narbonne 118
F-31077 Toulouse Cédex

Prof. J.L. Teyssier
Lab.des Radiations Ionis.
Université de Limoges
Rue A.Thomas 123
F-87060 Limoges Cédex

Contract no.: BIO-A-296-81-F

General subject of the contract:

Dosimetry of low doses by the electret and tracks effect. Application to the individual dosimetry of fast neutrons.

Description of research work:

At room temperature, random delocalized electric dipoles and free charges are included in organic polymeric matetials. So the result of macroscopic polarization is null. Yet if an high voltage is applied between both sides of the sample, a low electric polarization remains after the electric field was turned down. This phenomena shown by polymers and some other dielectrics is called "electret effect"; an electret is an electrostatic equivalent of a continuous magnet.
Experimental verifications show the possibility to use the electret depolarization in an ionizing particle field for dosimetry monitoring. The sensitivity is better than 0.1 mGy and the accuracy better than 10 % for X ray beams. Besides, the dosimeter readout system does not destroy the initial information.
Most of the present results are obtained in the biomedical dosimetry range and for two special cases such as X rays of clinical radiography with an energy included between 20 to 120 keV and gamma rays from a cobalt 60 source. Four study axis must be followed :
1° Measurements of very low doses for all important kinds of particule, with improvements of basic material to obtain a greater response. The polymer sensitivity used presently allows dose measurements lower than 1 mGy. This dose value corresponds to a loss of charge about 25 % for polyethylene electrets and more than 50 % for teflon electrets.
2° The energy response for various gamma-ray beams. An important remark is the low atomic number of neutral polyethylene close to that of biological tissues and the good correspondance of atomic numbers between teflon and bones.
3° Response for neutron/gamma mixed field and applications to fast neutron dosimetry. To discriminate the gamma dose and the neutron dose in a mixed field, it is foreseen to complete the electret surface potential measurements with a substrate analysis as a track detector material. Presently, but with a low efficiency, it must be detected backward the protons created by incident neutrons in hydrogenous compounds
4° Study of a routine electret readout system.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. P. Blasi; Prof. G. Moschini
Lab. Nazionali di Legnaro
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Contract no.: BIO-A-297-81-I

General subject of the contract:

Dosimetry and hazards of neutrons at energies between 15 and 50 MeV.

Description of research work:

Beams of neutrons with energies up to 50 MeV can be produced with the 16 MeV Tandem accelerator newly installed at Legnaro. This accelerator, in conjuction with the other Van de Graaff accelerators already working at Legnaro, provides a unique capability for studying radiobiological effects and dosimetry in a very wide energy region and in particular in the 15-50 MeV region where fundamental information is particularly sparse.

The objective of the research programme is twofold :

(1) Determination of the absorbed dose and quality of various neutron fields.

(2) Determination of RBE values, mainly from cell survival studies.

KERMA in air and absorbed dose values at selected points in a phantom will be measured for different neutron fields.

Computed values of KERMA (within the limitations of available cross-section data) will be compared with experimental measurements of KERMA to identify areas where further experimental work is required and to stimulate research by the appropriate specialists.

In carrying out KERMA measurements, the perturbations due to the finite size of the dosimeter will be evaluated both experimentally and theoretically.

The quality of the ration field will be studied by experimental and theoretical microdose distributions and subsequently by direct comparison with RBE values, determined experimentally in appropriate biochemical and biological inactivation studies.

The programme will be performed at the Laboratories of Legnaro in Italy in collaboration with the Department of Medical Biophysics at the University of Dundee, United Kingdom. The Dundee group will perform the theoretical computation of KERMA values, microdose distributions, dosimetric corection factors. The Legnaro group will perform the experimental measurements of dose, KERMA and microdose and, in conjuction with the Biological Departement of the University of Padova, the experimental RBE studies.

Intercomparison measurements among different European laboratories will be promoted by placing at their disposal the neutron facilities of the laboratories of the Legnaro group.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
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Contract no.: BIO-A-302-81-NL

General subject of the contract:

Neutron dosimetry instrumentation for radiation protection and radiobiology.

Description of research work:

The main purpose of the neutron dosimetry programme at TNO is to improve neutron dosimetry techniques for radiobiology and radiation protection. Although many physical data and measuring methods for neutrons have been published or elaborated in recent years, methods in personal neutron dosimetry and radiobiology are not completely satisfactory.

Specific attention will be given to the following subjects :

1. In radiobiological experiments with neutrons there exists always an unavoidable gamma component which must be measured separately. The accuracy and precision of mixed beam dosimetry still suffers from instrumental shortcomings. This includes practical problems, such as detector design, as well as theoretical problems with respect to detector response.
2. New regulations in radiation protection necessitates the knowledge of organ doses for relevant standardized exposure situations. For the determination of the dose distribution over the body, which is generally derived from measurements with TE ionization chambers, it is of importance to ascertain the effective point of measurement for neutron beams of different energies.
3. The development of detectors having a response proportional to dose equivalent for a wide range of radiation quality is required.
4. Considerable variations in dose distributions will occur at the interfaces of biological materials of different atomic composition such as bone - soft tissue, and air - soft tissue. The experimental studies of changes in quantity and quality will be extended over a wide range of neutron energies.

Examination of different types of commercial tissue equivalent (TE) ionization chambers has shown that the construction of the chambers is not uniform, and that there can be technical deficiencies e.g. inadequate positioning of central electrode, discontinuities in the inner wall of the chamber and imperfections in the connections of the guard electrode. There is still a need to construct TE ion chambers for radiobiology. For radiation protection applications, the sensitivity of the ion chambers has to be increased. The introduction of large size ion chambers (with volumes in excess of 1 liter) poses a number of practical problems, such as the validity of the Bragg-Gray relationship, the risk of incomplete ion collection and the inadequacy of TE gas flow through the chamber. In this programme on neutron

dosimetry instrumentation special attention will be given to the development of high pressure ion chambers or multiplication chambers. A thimble-type TE ionization chamber with a gas volume of about 1 cm^3 was constructed which can be operated at gas pressures of up to 10 MPa (100 bar). Measurements have been performed at pressures of up to 8 MPa of methane based muscle equivalent gas for ^{137}Cs gamma rays, 0.9 and 14.5 MeV neutrons. The reading of the ionization chamber at a fixed value of the collecting potential increases with increasing pressure, but this increase is limited by initial recombination. The saturation characteristics as well as the pressure dependence of the reading are dependent on the quality of the radiation and can be used to assess quality factors.
The response of TE ionization chambers will be studied as a function of gas pressure also for other types of gases with the aim of introducing systems with higher sensitivities. In addition, it is important to analyse the results in terms of Jaffé and Kara-Michantova/Lea theories to obtain insight in the relevant processes, which will facilitate the construction of a practical system for radiation protection purposes.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-434-81-UK

General subject of the contract:

Development of improved X-ray counters for assessment of plutonium in lungs.

Description of research work:

The Atomic Energy Research Establishment (AERE) has recently been concerned with the development of large area, low background proportional counters, with good resolution at energies up to and beyond 20 keV, for use in X-ray astronomy. The techniques developed in designing and producing these detectors have potential application in the detection of 13-20 keV X-rays from lung burdens of plutonium. Currently, scintillation detectors, rather than gas counters, are almost universally employed for these purposes because of their higher detection efficiency, despite of their greater background response. If the background could be reduced appreciably without substantial loss of efficiency, the limits of detection for lung deposits of plutonium would be reduced.

It appears entirely feasible to make a plutonium-monitoring detector, using the same techniques as those developed for X-ray astronomy, meeting the following specification:

Detection efficiency	:	over 90% at 20 keV
Area (2 detectors)	:	500 cm^2
Background rejection efficiency	:	99% of Co-60 gamma-ray induced background over 2 keV
Energy resolution	:	about 10% at 22 keV

The entire active volume of each detector would be protected on four sides by conventional anti-coincidence guard layers, and on the remaining two sides by end-guard cathodes; previous proportional counters used for these purposes (before they were generally superseded by 'phoswich' scintillation counters) have been protected on four sides only. Calculations suggest that an improvement in sensitivity by a factor of two or more, compared with what is possible using phoswich detectors, could be achieved.

The background performance and efficiency of a prototype, constructed during the first two years would be compared with those of existing counters, in the shielded enclosure of AERE's whole-body counter. It is expected that experience with this prototype, during the second year, would lead to further improvements in design which could be realized in a second-generation model, to be constructed in the last two years of the programme.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
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Contract no.: BIO-A-307-81-UK

General subject of the contract:

A study of the dosimetric applications of thermally stimulated exo-electron emission in beryllium oxide.

Description of research work:

Thermally stimulated exo-electron emission (TSEE) shows considerable potential for mixed field dosimetry. Measurements at the National Physical Laboratory using sintered beryllium oxide discs for fast neutron and gamma doses over the range 1 μGy to 1 mGy show reproducibility to better than 7 per cent, lack of dependence upon dose rate and absence of fading problems over a ten day period. With a graphite radiator the fast neutron dose sensitivity is 3 per cent only of the gamma sensitivity; for a hydrogenous radiator (polythene) the corresponding value is 45 per cent. The sensitivity to gamma-rays has been found to be constant between 100 keV and 2 MeV, and work elsewhere suggests that the neutron sensitivity varies little between 1 and 14 MeV.

It is proposed to investigate further the energy dependence of BeO dosimeters and to calibrate them in terms of the NPL standards of gamma dose and neutron fluence, and to make field trials of the measurement of mixed gamma-neutron fields by TSEE for personnel dosimetry and environmental monitoring.Other TSEE materials may be investigated using the NPL mass separator to produce samples by sputtering.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
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Contract no.: BIO-A-293-81-F

General subject of the contract:

Design and construction of a dosimetry system for the determination of the quality factor and the dose equivalent in neutron-gamma mixed fields.

Description of research work:

1. Microdosimetry studies conducted in conjunction with biology experiments have demonstrated the importance of ascertaining the D_G/D_N ratio in neutron/gamma mixed fields. The work carried out before was related to the design and construction of a technique for discriminating between neutron and gamma radiation and determining the quality factor of a mixed field by means of a Rossi proportional counter irradiated in neutron beams or placed in the penumbra of a collimator, for example. When associated with a conventional spectrometer (preamplifier, amplifer and multi-channel analyser), a counter of this type enables the gamma and neutron components of mixed radiations to be separated and the total dose measured. The use of this device has made it possible to study the variation of these two parameters and of the total dose equivalent in a collimated beam and in its penumbra. Nevertheless, these measurements take a fairly long time, require a sophisticated electronics system and cannot be immediately exploited.
   In the field of radiation protection today, the existing portable appliances, which measure the dose equivalent and generally use a moderator system, have a response that is more or less in line with the "QF/LET" relationship recommended by the ICRP. They are, moreover, relatively difficult to modify if changes in the values of the quality factor as a function of the linear energy transfer prove necessary.
   Lastly, most of these appliances do not take account of the gamma component of the beam under examination and are heavy and cumbersome.
2. The research work relating to the design and construction of a dosimetry system composed of a proportional counter and associated portable electronics will be conducted in the following manner :
   The first stage will consist in a methodological study of the apparatus with the aid of the existing spectrometer and will cover the following points :
   - optimization of the operational conditions of the measuring system;
   - determination of the detection limits and compatibility with the dose equivalent levels encountered in radiation protection.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Continuation contract no.: BIO-A-293-F

In the second stage, the design study of a portable prototype will be undertaken.
The assessment of the performance of the apparatus thus defined will be the subject of the third stage : to this end, comparative measurements will be made with the existing spectrometer in various configurations encountered around nuclear installations.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-288-81-D

General subject of the contract:

Biological effectiveness of incorporated radioactive nuclides and fast neutrons.

Description of research work:

With regard to tritium, the effectiveness after specific incorporation into DNA and after non-specific incorporation will be compared to Co-60-gamma rays for molecular and cellular endpoints. By considering the radiation energy deposition into DNA or into the cell nucleus the RBE values of tritium relative to Co-60 gamma rays will be evaluated for single strand breaks, double strand breaks, cell transformation and cell survival. It is the scope of these investigations to confirm that RBE values of molecular endpoints are different from those of cellular endpoints, and to obtain a RBE for tritium which is better correlated to late effects than the RBE for cell survival.

Considering the effects caused by I-125 it appears important to learn to which extent they result from the energy transfer of the emitted electrons or from charge accumulation and transmutation effects. The extreme radiation toxicity of I-125 in DNA is an interesting example of the possible radiation damage induced by Auger-electron cascades. Similar but smaller Auger-electron cascades are induced in the phosphorous of DNA and in the iodine of the thyroid by any external X- or gamma irradiation. It is the scope of this study to evaluate the relative contribution of the Auger-effect to the radiobiological effectiveness of photons.

Specific incorporation of tritium and iodine into DNA will be performed by application of the DNA precursor 3H-TdR, 3H-IUdR, 123-IUdR, 125-IUdR and 131-IUdR. The effects will be compared with those of homogeneously distributed nuclides, fast neutrons, and Co-60 gamma rays. Biological endpoints will be clone formation, induction and repair of DNA single- and double-strand breaks and cell transformation. Local effects of 3H- and iodine decay will be studied by measurements of strand breaks induced in isolated DNA. More information about the involved mechanisms is also expected from experiments under reduced oxygen tension and under the influence of radiosensitizers such as PNAP (paranitroacetophenone) and protective agents such as WR-1065 (M-(2-mercaptoethyl)-1,3-diamineopropane).

The corresponding dosimetric investigations will consider the primary electron spectra of the before mentioned radiation modalities, their secondary electron spectra, the energy distribution of the electrons depositing some or all of their energy in the sensitive volume, and the magnitudes of this energy deposition. The spectral distribution of the energy deposition of these electrons will be calculated in

collaboration with Dr. Paretzke, GSF Neuherberg. With regard to tritium particular attention will be paid to the dosimetric and microdosimetric differences of DNA-correlated interactions and random interactions between electron tracks and DNA.
In project two, it is proposed to develop a field instrument for the determination of the dose distributions in LET and the mean quality factors $\bar{Q}$ of mixed neutron-gamma fields of low intensity behind shielding walls. The scope of this investigation is at first the development of a method for the correct transformation of the microdosimetric spectra f(y) into LET distributions, second the test of simplified unfolding methods with regard to their ability of delivering a fair estimate of the LET distribution and of $\bar{Q}$, and finally the development of a field instrument using a microprocessor for the on-line processing of the collected ionization events. This will comprise the following investigations: Improvement of the method for the determination of dose fractions of the different radiation modalities of mixed neutron-gamma fields using microdosimetric spectra; Improved unfolding of the different radiation modalities by comparison with Monte Carlo investigations on the spectral energy deposition of the same neutron spectra. Transformation of D(y) spectra of individual radiation modalities into LET-spectra and evaluation of D(L) and $\bar{Q}$;Test of simplified transformation methods, such as the y-interval method, as to their ability of delivering a reasonable estimate of D(L) and $\bar{Q}$.
Selection and improvement of the best simplified method; Development of a field instrument using a logarithmic amplifier and a microprocessor for immediate presentation of D(y)-spectra, operator controlled autocalibration, and transformation into D(L).
In the third project the investigation on the depression of IUdR-incorporation after whole-body irradiation will be continued and the experimental conditions which are essential for the detection of this effect will be elucidated. The aim of these studies is the development of a biological dosimeter for the low dose region, where chromosome aberration counting is no longer appropriate.
The mechanisms underlying this effect are not fully understood but the biological results and microdosimetric analysis indicate that at the smallest doses the effect is due to fundamental changes of cellular organelles which control intercellular mechanisms, such as the cellular membranes. Experiments will be carried out to further elucidate the radiation mechanisms, in particular the relative contribution of serum effects and the role of intercellular contacts. The effect will be investigated for different cell systems covering the transition from isolated cells to spheroids and then to animals. The possible use of this effect for low dose biological dosimetry will be studied by repeating the measurements with dogs in collaboration with Brookhaven National Laboratory then by applying the test system to radiotherapy patients of the institute.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-287-81-D

General subject of the contract:

Determination of effective organ doses.

Description of research work:

Within the framework of this research programme, two aspects will be investigated.

1. Physical Aspects of Radiation Effectiveness. Radiation risk by occupational neutron exposure at several steps in the nuclear energy production cycle is still a matter of dispute. A unique quality factor, as defined on the basis of the average LET of the charged particle spectra within an organ, does not seem to provide a good measure of relative organ risks. Furthermore a constant quality factor for photons seems unreasonable. Based upon the results of the recent programme, experimental and theoretical investigations on radiation physics and microdosimetry will be continued for an improved specification of radiation quality.

2. Organ and Phantom Models Improvement and Calculation of Effective Organ Doses. Anthropomorphous phantom models provide a commonly acceptable basis for the specification of organ doses. Two improvements of the currently used heterogeneous MIRD-5 model are necessary :

- the modification of organ geometries in connection with a special sex-specific organ weighted dose concept, as the effective dose equivalent in an unique phantom does not seem to be adequate for risk determination in medical exposure,
- the refinement of the crude bone model for improved evaluation of dose deposition in the red bone marrow, trabecular bone and tissue layers at the bone surface.

The organ doses are determined by means of Monte Carlo calculations, resulting in spectral photon or neutron fluences inside organ meshes. From these the kermas are derived and taken for the equilibrium dose. This is wrong at interfaces of materials of different density or atomic composition, which becomes crucial in case of a refined non homogeneous bone model. For neutrons the kerma is irrelevant without the quality factor. Therefore the existing codes have to be extended for the transport of the secondary particles.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-A-284-80-D

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General subject of the contract:

Investigation of different dose equivalent quantities in phantoms externally irradiated with photons, electrons and neutrons.

Description of research work:

A number of new concepts for radiation protection have recently been proposed and discussed, e.g. the deep dose equivalent index $H_{I,d}$, the mean dose equivalent in a sphere H_K, the dose equivalent ceiling H_C, the depth dose equivalent H_d for penetrating radiation, on the one hand, and the shallow dose equivalent dose index $H_{I,s}$ and skin dose equivalent H_s for non-penetrating radiation on the other hand. The applicants have recently developed a theory in which a direct mathematical relationship is established between the new concepts recommended by the ICRP, for which dose limit values are laid down (e.g. the effective dose equivalent), and the reading of various types of detector or dosemeter. It is expected that this theory will influence the choice of the practical concepts to be used in future.

New internationally uniform concepts must be introduced on a metrologically sound basis for all types of radiation. Since the unit of a new quantity cannot be realized at short notice, if at all, by primary standard measuring equipment, the quantity must be related by conversion factors to other quantities (so called calibration quantities) for which standard measuring equipment exists. Examples of such calibration quantities are the exposure ion dose for photons and the fluence for neutrons. The relationship to conventional dose measurements or radiation field quantities can be determined by calculation (e.g. by the Monte Carlo method) or experimentally. Both procedures have advantages and disadvantages and complement one another.

The research programme to be carried out falls into four parts :

(1) Further development of the above-mentioned theory.
The theory has been developed up to now on the assumption of a two-dimensional distribution of remote external radiation sources. Further considerations have shown that it should be possible to generalize this theory for the case of any three dimensional distributions of remote external radiation sources. The generalization of the theory is being carefully studied and put into effect and a new operational quantity is proposed for radiation protection.

(2) Monte Carlo calculations
The theory cannot be used for practical detector development, however, unless the phantom response matrix is known in numerical terms. The second part of the research proposal is therefore

concerned with the exact determination of this parameter for the case of neutron irradiation. This is certainly possible for the case of the two-dimensional theory but the aim is to determine the matrix for the case of the three-dimensional theory also.

For this purpose, an existing computer programme by which the phantom response matrix can be calculated, using the Monte Carlo method, is being revised and further developed. Other Monte Carlo calculation procedures are being applied for a sphere phantom with photon, electron and neutron radiation.

(3) Measurements for photon and electron radiation.
Existing calculations show the limits of the calculation procedure. The statistical uncertainties cannot be reduced to satisfactory levels without considerable additional work. Systematic uncertainties are difficult to estimate, since they depend on the model and on the data used. It is therefore desirable to back up the calculations by experimental work. The above-mentioned conversion factors and the influence of the angular distribution of incident radiation on measurements in the sphere phantom will be experimentally determined and compared with the calculated data. Further points in the programme are the development of design principles for radiation protection dosimeters the response of which in units of the new quantities is as far as possible independent of energy (and, where appropriate, independent of the direction of radiation incidence), the development of calibration procedures for area monitoring dosemeters and personal dosemeters with a view to the use of new concepts of measurement for all types of radiation and all energies.

(4) Measurements for neutron irradiation.
In order to check the reliability of the calculations mentioned under heading 2, dosimetry and/or microdosimetry measurements of the depth dose distributions in a homogeneous phantom of simple geometric form are being compared with the corresponding calculations. Further experiments aim at improving the neutron cross-section basis of the Monte Carlo code. The essential neutron scattering cross-section for the elements carbon and oxygen, the main components of tissue, are being measured for a set of different energies between 8 and 15 MeV.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-286-81-D

General subject of the contract:

Microdosimetric studies and risk assessment for late somatic effects.

Description of research work:

Research aimed at further elucidation of the relative biological effectiveness of different ionizing radiations and its dependence on absorbed dose will be continued and extended. Special emphasis will be on radiation carcinogenesis at low dose levels relevant in radiation protection, and on the problem of linearity of dose-effect relations in this dose range. Microdosimetric and biophysical and epidemiological investigations will be performed that are required for the stated purpose.

Project one will continue the present work to obtain distributions of specific energy, their mean values, and the proximity functions for regions of magnitude 1 nm up to several μm. These calculations will relate to electrons, to neutrons, and to heavy ions and will provide data required in the analysis of RBE and of risk factors. Efforts will be made to develop further the concepts of microdosimetry and to link them more closely to the concepts and the numerical methods of classical dosimetry.

Project two will be directed towards a systematic analysis of the neoplastic response to gamma- and neutron irradiation in the Japanese atom bomb survivors. This is to be an epidemiological investigation based on considerations of theoretical radiobiology and - depending on the outcome of the revision of the dosimetry - also concerned with the investigation of RBE as a function the level of effect. The aim is to supplement the findings of RERF by results not based on particular models but derivedfrom non-parametric analysis with maximum likelihood methods. The project will be a joint effort with Rossi et al. at Columbia University, New York, and members of RERF including, in particular, Dr. Kato, chief of epidemiology at RERF. Input data will be provided and extended within the next years, and two research projects have been formulated and have been officially approved at RERF. The analysis will utilize actual kerma estimates for the life study sample (LSS) instead of the rough classification into certain kerma intervals presently employed. Similar non-linear optimization techniques as in project one will be employed.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-A-310-81-UK

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General subject of the contract:

Application of lyoluminescence to radiation dosimetry and radiation protection.

Description of research work:

As a result of continous development of lyoluminescence dosimetry it is now known that lyoluminescent phosphors respond to exposure to gamma and X-rays as well as to electrons and heavy charged particles. It became thus possible to apply lyoluminescent dosimetry (LLD) to the needs of radiotherapy and to some industrial processes associated with radiation food processing.

The main features of lyoluminescence as a basis of technique of radiation dosimetry are :

- very good tissue equivalence can be achieved by a choice of LL phosphors from a variety of saccharides, amino acids and proteins. This equivalence is retained for a broad range of energies of radiation and for all types of interaction.
- very low cost of LLD phosphors and moderate cost of readout equipment.

It is proposed to pursue research in new applications of lyoluminescence and at the same time to continue research in the mechanisms underlying the phenomenon. At present the sensitivity of LLD is limited by the presence of "chemical" background to about 0.01-0.03 Gy. Theoretical considerations lead to a conclusion that in the absence of spurious background arising from impurities in phosphors the amount of available light will set the limits of detection to about 10^{-4} Gy for a 25 mg sample. It is thus proposed to study the means of reduction of "chemical" background by development of adequate preparative and purification techniques, covering both the LL phosphors and the sensitizers (enhancers).

The pratical aim of this research is to lower the measured radiation dose, so that lyoluminescence could be employed for purpose of accident dosimetry. It has been shown that radioactive nuclides incorporated into LLD phosphors are producing an internal dose, which can be easily measured, provided that a calibration facility is available. This opens the way for measurement of dose from low energy beta emitters, including tritium and sulphur-35. Furthermore, dose from Auger electron emitters produces measurable effects in LL phosphors. This dose can be separated from dose delivered by long range radiations by a use of phosphors of varying geometry. It is proposed to study in detail radiation effects produced by low energy beta and Auger emitters incorporated in organic biochemicals.

The range of materials which can be studied in this way is very broad, including not only sugars, amino acids and proteins but also nucleotides (DNA and RNA). Calibration of the internal dose will be provided by external sources of low energy electrons and soft X-rays.
It is expected to employ lyoluminescence technique to study stimulated Auger emission from biochemicals incorporating heavy atoms (e.g. iodine, bromine, selenium etc.). When such materials are irradiated with a beam of X-rays with energy near the K-absorption edge, stimulated Auger electron emission takes place, which locally enhances the radiation dose. This effect, postulated by Feinendegen, may play an important role in radiosensitization of cells and tissues and lyoluminescence offers a simple way of observation of radiation effects in biomolecules in solid state caused by low energy electrons. It will be necessary to construct an apparatus for quasi-monoenergetic X-ray irradiations. The use of lyoluminescence technique will be extended to cover dose deposited by short lived nuclides, particularly those used in nuclear medicine, in solids of composition corresponding to soft and calcified tissues. This will provide information on the direct action of radiation, which is still incomplete, particularly in the case of positron emitters.
If available, such short lived nuclides as C-11 and F-18 will be included in the investigations.
It has been observed that various plastics, man made fibres fragments of dried tissues like skin flakes, hair, show lyoluminescent response. Such materials are natural dosimeters which could be used for approximate determination of absorbed dose and its distribution in case of nuclear accidents, radiation accidents, inadvertent exposures. Practical implementation of this technique requires formulation of efficient solvents, because low solubility rate of above mentioned materials seriously limits the minimum measurable dose. Dose calibration can be achieved by adding a known dose to the dose previously received by material. Improvement and better understanding of the enhancers (sensitizers) will be needed as a necessary preliminary.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-463-81-UK

General subject of the contract:

Dosimetry and microdosimetry of intermediate energy neutrons.

Description of research work:

Microdosimetry techniques will be developed for the measurement of dose and quality of intermediate energy neutrons at energies between 10 keV and 100 keV.

A microdosimeter will be designed specifically for use in neutron fields where there is a significant intermediate energy neutron component. The main technical problems to be solved are :

a) to separate events induced by the fast neutron component and gamma-ray fields, which are likely to be present in practice, from the events of interest induced by the intermediate energy neutrons;

b) to minimise field perturbation whilst maintaining adequate dose sensitivity;

c) to maximise the detection sensitivity for the low energy recoil charged particles. It is anticipated that these problems can be surmounted by the novel application of fast coincidence anti-coincidence techniques to a co-axial double cylindrical detector fitted with a thin dividing wall which will be designed to enhance selectivity between recoils initiated by fast and intermediate energy neutrons.

In parallel with the development of the microdosimeter certain other relevant physical information will be amassed. A computer programme will be prepared for calculation of kerma factors and microdose spectra for various intermediate energy neutron spectrum shapes. The neutron spectra will be measured using threshold detectors and other standard techniques.

Information on quantities such as drift velocity, agitation velocity, recombination and attachment coefficients which influence electron and ion transport in gas-filled proportional counters will be studied with the object of optimising counter performance.

Upon successful development of the instrument, microdose spectra will be recorded in field trials at shielded positions around power reactors and fuel processing plants to assess dose equivalents.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-298-81-I

General subject of the contract:

Microdosimetric studies of radiological effects at low doses.

Description of research work:

The principal objective of radiation protection is the achievement and maintenance of appropriately safe conditions for justified activities involving potential human exposure. In this sense, although directed primarily to man, radiation protection cannot rest only on the scarce evidence existing so far on detrimental effects to man, but must also take advantage of the much larger experience gained through radiobiological studies on a variety of biological systems, using similarities of behaviours between the responses of the different systems to radiation. In other words, a certain process of unification in the interpretation of basic mechanisms of action is likely to produce, on the long range, some important results also for radiation protection, as for other branches of science, by establishing unambiguous correlations between the physical structure of radiation and the modalities of interaction on the one side, and on the other side the aptitude of radiation to produce irreversible damage in the irradiated tissues, and eventually cause detriment to the individuals and their progeny. In order to avoid unsafe procedures of extrapolation to low doses, it becomes essential to work out the hypothesis of similarity and, by using also the information obtained from highly radiosensitive systems, try to assess whether some observed trends, for instance in the dose-effect or in the dose-RBE relationships, might realistically be adopted as a more general feature for low dose irradiations. In particular, although the hypothesis of linearity is generally adopted for assessing risk estimates at low doses, the results of recent experiments with neutrons suggest that for some end-points on highly radiosensitive biological systems the effectiveness of fast neutrons might be higher at low doses, around one rad. This fact indicates the necessity of more evidence substantiating the type of extrapolation adopted to estimate the risks associated with neutron exposures at low dose levels. Better evidence might become necessary to clarify the problem of whether an increasing RBE with decreasing neutron dose result only from a decreased effectiveness of the reference radiation or might also correspond to an increased effectiveness of neutrons at low dose. The solution of this problem may have important consequences for the values of Q-factors to be used in radiation protection.

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Continuation contract no.: BIO-A-298-81-I

It has been foreseen by microdosimetry that the biological effectiveness of radiation is influenced in a fairly predictable manner by the characteristics of the energy deposition pattern in the irradiated tissues. Significant changes in neutron effectiveness can be expected, therefore, and are in fact observed, when neutron energy is varied throughout the energy region up to, say, 20 MeV. In order to generalize these conclusions the comparison between microdosimetric predictions and results of radiobiological tests has to be extended to the largest possible choice of neutron beams. Microdosimetric investigation of neutron beams actually employed remains, therefore, of a primary validity. In this context the problem of biological response saturation with increasing radiation LET must be carefully investigated.

Finally it has to be observed that all research areas indicated so far require a fairly well established neutron and photon dosimetry. Existing devices are especially well suitable in the intermediate-to-low dose region. It would be, therefore, worthwhile to extend the available technology to cover the region of doses around and below one rad, which is of the most concern for radiation protection. Accordingly, the research program will cover the following items :

a) Measurement of energy deposition spectra.

Microdosimetric measurements have to be carried out for beam qualities utilized for the biological experiments, to provide the reference quantities to be used for the interpretation of biological results.

b) Measurements of biological responses to low-dose irradiations.

Radiobiological tests will be carried out with fast neutrons and photons of various energies on different systems including in vitro cell cultures, roots and the mouse. Neutron irradiations will cover the energy between 0.2 and 18 MeV. Planned experiments will extend existing studies and begin new programmes aimed to investigating dose-effect relationships for endpoint of direct interest to radiation protection, such as mutagenic, carcinogenic and teratogenic aptitude of radiation, and that can be directly used for microdosimetric considerations, such as formation of micronuclei and other chromosomal aberrations.

c) Application of microdosimetric models to interpretation of radiobiological data.

The results of radiobiological experiments will be analized using existing models of radiation action in order to verify their validity. The possibility of disposing of a fairly large set of data, all obtained at this same laboratory, will make such an endeavour more meaningful. The problem of the saturation of biological action for high LET radiation will need further attention.

d) Neutron dosimetry. The extension of the utilization of existing techniques, mainly ionization chambers, to low levels of dose and low dose-rates might represent a long-range cooperative effort together with other laboratories.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-A-299-81-I

General subject of the contract:

Personal dosimetry and area monitoring.

Description of research work:

New detecting methods and apparatus have been developed at CNEN with the major mission-goal of registering low doses of high-quality-factor radiations (high-LET particles and neutrons). The trait common to these different registration techniques is the use of the high energy depositions occurring along nuclear tracks in solid dielectrics, to initiate catastrophic events at track sites. These avalanche-types of processes are such that they can be easily detected by simple read-out techniques. The simplicity of these new detection systems, their very high sensitivity and their ability to discriminate against large fluxes of low-LET radiations make them very promising for the assessment of the low fast neutron doses encountered in personnel dosimetry and area monitoring and the detection of natural and man-made alpha-emitting radionuclides.

These detectors can be divided in two categories : a) Electrochemically etched damage track detectors : b) Thin-film breakdown counters. The thin-film detectors can be considered to complement the damage track registration. The damage tracks are in practice stored for an indefinite length of time, while the thin-film capacitors present fast-time reponse. The damage track detectors have been extensively investigated in the past two decades and various applications are proposed in this contract. By contrast, research on thin-film detectors has just started and a lot of development work is still required.

a) Electrochemically etched damage track detectors

Electrochemical etching makes possible to enlarge nuclear tracks in solid dielectrics up to macroscopic size, so that the scanning of large detector areas (necessary for the detection of low neutron doses and low alpha activities) is greatly facilitated. The most interesting plastic material is the thermosetting polymer CR-39, because of its high radiation sensitivity and excellent properties for the electrochemical etching. Results already obtained have shown that polyethylene coated CR-39 foils from American Acrylics detect neutrons with energy down to 100 keV with conveniently high signal-to-noise ratio. These results suggest that, at last, a convenient personal fast neutron dosimeter could be developed. To this end a systematic investigation is planned on CR-39 detector response versus energy, angle of incidence and dose of fast neutrons. To obtain a personal dosimeter sensitive to neutrons in the entire energy range, composite

albedo track dosimeters will be analysed. For the detection of alpha particles, the CR-39 thermosetting polymers present unique characteristics for low-activity measurements of transuranic elements. Since the minimum detectable alpha activity for long-lived elements both in the man and environment is often uncomfortably high, investigations will be made to further improve the signal-to-noise ratio of the CR-39 foils properly manufactured to obtain low-alpha background. In practice the track detectors provide only "gross-alpha" determination, while alpha spectrometry is necessary for the identification of the alpha emitters, which may have differences of 5 order of magnitude in the maximum permissible concentrations. A new method for alpha spectrometry will be investigated which is based on the measurements of track-spot diameters.

For monitoring the routine personal neutron doses and the individual exposures to alpha-emitting radionuclides, difficulties arise for the etching and counting of great many samples with the present methods and apparatus for electrochemical etching. To overcome these limitations, a new electrochemical etching method will be further investigated. This method makes possible to electrochemically etch plastic foils with thicknesses as low as 10 microns so that they can be conveniently scanned by the spark counter.

b) Thin-film breakdown counter

The thin-film detectors, based on the observation of breakdowns induced by high-LET particles, provide simple heavy-ion and neutron dosimeters with the only requirements of a thin-film capacitor, a power supply of a few tens of Volts and a scaler. With such real-time dosimeters, large scale field survey, area surveillance and personal alarm dosimetry could be greatly facilitated. So far these detectors have been studied mainly with silicon-dioxide capacitors. Investigations will be made to study the response of other types of thin-films. Detector thicknesses from 200 A up to tens of thousands of A will be used. Finally the response of the thin-film counters will be analysed versus the energy depositions from charged particles with different masses and energies for a variety of thin films.

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Head(s) of research teams(s):

Contract no.: BIO-A-289-81-D

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General subject of the contract:

In vitro and in vivo investigations of radiation induced chromosome damages and microdosimetric techniques for neutrons and high-LET radiations.

Description of research work:

The objective of the first part of the research programme is to detect the relation of aberrations, radicals and dose and to contribute to the understanding of the primary mechanisms of radiation damage. Therefore, investigations of radicals induced in frozen solutions of chromatin with and without histones and in solutions of amino acids and peptides will be extended to neutron radiation. The behaviour of radicals at increasing temperature which is expected to depend on the ionization density will also be observed. Correlated studies of the chromosome damage in lymphocytes will be carried out. The radicals induced in cell nucleus constituents (DNA, proteins and water respectively) will be determined to evaluate the relation of radicals, chromosome damage, cell survival and doses and to compare the effects of densely and sparsely ionizing radiation. To improve the analysis of EPR-spectra and radicals the EPR equipment used will be extended to enable pulsed measurements (see below).

In order to study the relevance of the in-vitro investigations of chromosome damage with regard to their applicability in radiation protection their results will be compared with in vivo results. Therefore the mean whole body doses and the chromosome damage of radiologically examined infants, children and adults will be investigated (heart catheterization). Blood samples of adults are studied immediately and at an appropriate time after radiological examination to discriminate the rates of damage in cells irradiated within and outside the lymph cells producing organs. With children and infants the studies will be repeated at intervals of one or more years to test the fate of the chromosome damage. The examination of diagnostic patients contributes to the unsolved problems of the dose effect relationship in the low dose range. Examining also radiotherapy patients the high dose range of the dose effect curve will be covered. In order to find out if the rate of damage depends on the irradiated part of the body, preferably patients irradiated either in the thorax or in the thorax and abdomen will be included in the study.

In the second part of the programme, the detailed information obtained in experimental microdosimetry will be used to determine physical data of the radiation field under investigation which are relevant for the solution of practical problems in neutron and high-LET dosimetry and in dosimetry protection purposes. Method of separating dose contributions

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from different primary beam components will include the study of coincidence techniques between Rossi-counters and other detectors in order to electronically discriminate events from different primary particles. Methods to determine neutron energies from microdosimetric spectra will be investigated. Such determinations are of relevance to neutron dosimetry because the measurements can be carried out at almost any point of interest, for instance in a phantom, and because kerma values and neutron sensitivities of non-hydrogeneous detectors are dependent on neutron energy. Microdosimetric spectra measured in free air for monoenergenetic neutrons up to 20 MeV accompanied by measurements of the neutron fluence will be compared to corresponding calculated spectra. These comparisons will allow to draw conclusions on the neutron cross sections in carbon and oxygen which are of great importance for the kerma values and thus for neutron dosimetry at high neutron energies. Microdosimetric measurements will be performed in a phantom for monoenergetic neutrons and related to Monte Carlo calculations for the development of a method to determine the effective dose equivalent. They will be performed in correlation with radiobiological experiments with radiations of widely varying radiation qualities.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-433-81-F

General subject of the contract:

Device for dose equivalent measurement in mixed fields.

Description of research work:

Microdosimetry has made considerable progress in recent years, and the so-called Rossi counters are routinely applied for research purpose and also in all detailed analyses relevant to accidental exposures in unknown radiation fields, or in radiation fields with only partially known characteristics. It is therefore surprising that they are not routinely applied for monitoring purposes. In principle they permit a ready determination of the dose equivalent and the quality factor even at extremely small doses or dose rates.

Dose equivalent meters with Rossi counters have only been built for a special application, namely monitoring in the Supersonic Transport, and as a prototype instrument in Brookhaven National Laboratory. Both instruments are bulky and not quite suitable for general application.

A modern version that utilizes state of the art TE proportional counters and recently available microelectronics should allow to develop an area monitoring portable instrument.

The most efficient estimates of D, H and Q are obtained from two simple sums of weighted event sizes. ICRU bases the definition of Q on LET, however this has been merely a practical matter in order to keep the definitions as simple as possible. The associated random variables y or z are more directly related to the biological action of ionizing radiations. There has been a recent proposal to define Q in terms of the microdosimetric quantity y rather than in terms of LET. However this is largely a conceptual problem. Even if the definition of Q in terms of LET will be retained in the future, one can substitute LET by event size. The resulting estimate of Q will probably be slightly affected by this substitution.

In particular it will be unnecessary to apply the numerical method that is conventionally utilized to derive formally a LET-spectrum from a pulse height distribution.

If one states that event sizes measured in a TE proportional counter can be used instead of LET to determine the quality factor, this counter is therefore a suitable detector. But the development of a counter, more suitable than the somewhat unreliable EGG instruments that are presently commercially available is necessary.

The electronics could include a logarithmic amplifier. The exact characteristics of the amplifier are not critical, since the relation pulse height versus energy imparted can readily be accounted for in the

microprocessor used with the instrument. However, stability is required, and this may lead to the choice of a pair of linear amplifiers that covers the low pulse height and the high pulse height range. The total range of pulse heights to be covered extends over more than 3 orders of magnitude.
A microprocessor will be very suitable for processing of the individual pulses registered in the instrument. It will also provide the storage of results. These include absorbed dose, dose equivalent and quality factor. A sum distribution of event sizes, or dose distribution in quality factor, can readily be stored in addition. Its knowledge may be profitable under certain conditions.
These three studies and realization will be treated simultaneously so that

- a laboratory prototype will be made at the end of 1981 and tested in 1982;
- the first industrial prototype will be realized at the end of 1982 or the beginning of 1983;
- this prototype will be tested and eventually modified during 1983.

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Head(s) of research teams(s):

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Contract no.: BIO-A-305-81-UK

General subject of the contract:

Radiation dosimetry and spectrometry.

Description of research work:

Neutron dosimetry is becoming more important in the Community with increased processing of high burn-up nuclear fuels containing plutonium and curium isotopes. The first project aims at extending the energy range for neutron spectrometry down to a few keV. A neutron spectrometer will be developed based on a set of spherical hydrogen proportional counters. Two-parameter data accumulation (pulse amplitude and rise-time) will be used if this is found to be necessary. This system will cover the neutron energy range from 5 keV to 1 MeV. The organic scintillator already developed for in-phantom spectrometry covers the range 1 to 7 MeV and will be further developed by the use of a different scintillator and iterative and least-squares unfolding techniques. The combined system will thus cover the energy range from 5 keV to 7 MeV (and possibly higher), and will be used for two purposes :

1. To set up and characterise reference radiation fields for performance tests on neutron dose-meters and Bonner sphere systems. The reference fields will be based on a ^{252}Cf source with a set of shields.
2. Neutron spectrometry in the field, i.e. at about maximum permissible flux levels, at reactor and fuel processing sites.

For the spectrometry of reference radiation fields a set of four counters (type SP2, 4 cm diameter) will be used, filled with hydrogen at various pressures from 0.5 to 10 atmospheres. EHT's up to 3.6 kV will be required. For the greater sensitivity required in environmental spectrometry four counters (type SP6, 15 cm diameter) filled with hydrogen will be used. There may be problems with the high EHT required for the highest pressure filling, and it may hence be necessary to resort to a hydrogen/argon mixture or a cylindrical counter. It may be possible to construct the response functions analytically, but in any case they will have to be checked experimentally at certain energies, using mono-energetic neutrons. To unfold the spectra from the pulse height distributions existing computer programs such as SPEC4 and RADAK will be used.

The second project covers the investigations of new types of personal dosimeters. A dosimeter is required to replace the nuclear track emulsion which is subject to fading, has an uncertain threshold in

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Continuation contract no.: BIO-A-305-81-UK

operational use, and in which the tracks are difficult to count. The project forms a logical extension of the neptunium fission-foil dosimetry system, which because of high cost and radiotoxicity, cannot be brought into widespread use.

Plastics and etching techniques will be studied in an attempt to produce a sufficiently sensitive neutron personnel dosimeter in which the tracks of direct recoil ions (H, C, N, O) are counted in the plastic material after chemical processing. The dosimeter will need to have a low neutron energy threshold (<0.5 MeV) and a low background. At present CR-39 plastic and pre-etching followed by electrochemical etching appear to be the most promising combination to achieve this and to produce tracks which can be easily counted using a microscope.

Irradiations of the plastics using monoenergetic neutrons (100 keV-14 MeV) will be undertaken, optimum processing (temperature, time, frequency, voltage etc.) will be investigated and the particle tracks viewed optically. Effects of hydrogenous and non-hydrogenous radiators positioned adjacent to the detector will be investigated. Environmental effects on the detector will be studied. The ability of the Quantimet Image Analysing Computer to separate tracks from background imperfections will be determined, and other counting methods may be sought.

The technical description of a closely related research programme on the examination of the photo-transfer process in TLD materials is given under contract BIO-A-459-81-UK.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-A-306-81-UK

General subject of the contract:

Studies in microdosimetry, cellular radiobiology and track structure.

Description of research work:

The basis of all radiological protection norms and risk estimates is a study of the biological effects of radiation. This also requires a knowledge of the basic physics of the interactions of radiation with matter. Starting with survival and mutation data from mammalian cells, microdosimetric models have been used to calculate for different radiations various parameters such as RBE, interaction diameters, etc. as a function of dose and dose-rate. Fundamental to a full understanding of the biological effects is the study of the physical track structure of ionizing radiations. Thus the aim of this programme is to bring together the work on mutation and survival in mammalian cells and the study of track structure in a low pressure cloud chamber through the theoretical modelling using microdosimetric techniques. These techniques can then be used to calculate risk for various radiations and to establish RBE's and hence quality factors. More details about a research programme on theoretical microdosimetry are given in the technical description of contract BIO-A-460-81-UK. This programme focusses on cellular radiobiology and studies on track parameters of ionizing radiations.

1. Cellular radiobiology
   Experimental studies will be carried out on inactivation, somatic mutation induction, oncogenic transformation and chromosome aberration produced in cultured mammalian cells by various ionizing radiations, in particular, by gamma radiation, fast neutrons, tritiated water and incorporated radionuclides (I-125 and P-32). The cells available initially are mouse lymphoma cells (type L5178Y) primary human fibroblasts and Chinese hamster cells (type V79), but trial experiments will be carried out with a human lymphoblast line and, depending on the results, work may be concentrated on this line. Particular attention will be given to the use of synchrony, dose fractionation, various culture conditions and chemical sensitisers and protectors to isolate the component mechanisms of response and to look for inter-relationships between the different end points. Models of radiation action derived from microdosimetry and target theory will be fitted to each component of response, with the particular aim of predicting the response at very low dose and dose-rate.
2. Track parameters of ionizing radiations
   In the low pressure cloud chamber the spatial distribution of

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Continuation contract no.: BIO-A-306-81-UK

ionizations can be determined experimentally since droplets are formed on individual ions. The project will be a continuation of current work in which photographs have been obtained of tracks produced by alpha particles and low-energy x-rays in various gas mixtures including a tissue equivalent gas and pure water vapour. From the photographs the coordinates of the droplets are obtained for futher analysis using an image analysing computer.

Techniques for obtaining track parameters and distributions of relevance to microdosimetry and radiobiological modelling will be further developed. In the case of alpha particles these will be obtained from the two dimensional coordinates since matching of orthogonal views to obtain three dimensional coordinates is not generally possible due to the high density of ionizations. Distributions will be compared, where possible, (generally for water vapour) with those obtained theoretically by other workers. The effect of diffusion in the chamber will be studied further and experimental parameters will be optimised to minimise the effect of diffusion. The chamber will be adapted so that accelerator produced particles, especially protons, can be studied.

This study will provide a detailed analysis of tracks produced by low energy x-rays, alpha particles and protons in water vapour or simple gas mixtures for comparison with theoretical studies and in tissue-equivalent mixtures for applications in microdosimetry and radiobiology.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-A-459-81-UK

General subject of the contract:

Radiation dosimetry and spectrometry

Description of research work:

This research programme is closely linked to the programme described under contract no. BIO-A-305-81-UK. The latter programme includes two projects, a first one regarding neutron spectrometry and a second one regarding the development of a fast neutron personal dosimeter using recoil ions.

The exposure of phosphors at elevated temperature to UV light is a technique used in many laboratories to re-estimate doses measured by LiF phosphors. It has been shown that the response of peaks 6 and 7 in LiF TL phosphor depends on the LET of the radiation. Preliminary work at Harwell indicates that there is also a LET effect on the deeper traps involved in the UV photo transfer process. Further research is necessary to understand this effect. This will enable accurate interpretations of re-estimated readings to be made.

Glow curve analyses will be performed using a Multi Channel Analyser and a computer to investigate the effects of such factors as (1) the annealing conditions, especially cooling rate, (2) the type of initial radiation (e.g. neutrons, photons etc) and its LET and (3) the UV irradiation conditions. These factors will affect such parameters as the re-estimation efficiency, the sensitivity to UV light of phosphors not exposed to ionizing radiation and also the supralinearity of the peaks in the "re-estimated" glow curve. Explanations will be sought for the increased standard deviations of readings from phosphors exposed to UV light compared to the standard deviations obtained when the same phosphors are exposed to gamma rays. Initially work will be limited to ^{6}LiF and ^{7}LiF extruded dosimeters but will be extended later to include other phosphors such as CaF:Dy and CaF:Tm. It is hoped that the knowledge gained from the research can be used to extend the uses of phosphors. It might also be possible to increase the maximum measurable dose using this re-estimation system.

In carrying out this programme, proper attention will be paid to similar work in progress elsewhere and collaboration will be sought with other laboratories in the UK and Europe.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-A-292-81-F

General subject of the contract:

Dosimetry of neutrons around installations using or processing nuclear fuel.

Description of research work:

The proposed research is divided into two projects concerned with the development of neutron dosimeters adapted to various installations (PWR-fast breeders) using or processing nuclear fuel. They are the logical consequence of work which has been in progress for many years at the Department of Protection, Fontenay-aux-Roses and Cadarache on neutron dosimetry and spectrometry. This work has revealed the existence of relatively high neutron dose rates, particularly in power reactors, and indicates a need for dosimeters adapted to such environments.

The research would, at the same time, relate to the development of a personal dosimeter and of environmental dosimeters intended for the calibration of the personal dosimeters and for the measurement of radiation fields for radiological protection purposes.

Project one considers the design and construction of individual neutron dosimeters.

(a) Disadvantage of using fast neutron detectors
Various dosimetry techniques, in particular the use of recoil protons, enable fast neutron doses to be determined with an acceptable degree of accuracy but are unsuitable for the detection of neutrons of energy less than 1 MeV. Many sources, especially those surrounded by shielding, provide environments very rich in neutrons in the intermediate energy range which are not detected by such dosemeters : experience has shown that the nuclear emulsions currently in use give zero or inaccurate readings for such sources.

(b) Disadvantage of using thermal neutron detectors
Since thermal neutrons are easiest to detect, many types of dosimeters have been developed which are based on the measurement of thermal neutrons delayed and back scattered by the wearer's body (albedo dosimeter). Such devices have unfortunately proved to be hypersensitive to thermal neutrons, and, in order to obtain acceptable results, it is necessary to provide a dosimeter with multiple detectors, the interpretation of which is tricky and the characteristics rather dissapointing.

(c) Use of epithermal neutron detectors
Measurements of power station environments have shown that there is fairly good proportionality between the epithermal neutron flux density and the total dose rate and that detectors particularly sensitive to these neutrons energies (3" or 4.2" moderating sphere,

Uranium-235 fission chamber under Cadmium) give an indication consistently proportional to the dose. For this study, therefore, the use is proposed of detectors which record only epithermal neutrons. In this way very simple systems consisting of a single detection element can be designed.

The detection principle for a personal dosimeter could be either thermoluminescence or ionography.

The studies will proceed along these two lines :

- Thermoluminescence has the advantage of easy application. It is moreover already used for the measurement of gamma-doses. Sensitivity to gamma-rays means that the neutron dose can be determined only from difference between the data from a TLD which is highly sensitive to neutrons (Li^6F) and those from a TLD which is insensitive to neutrons (Li^7F). This same sensitivity to gamma rays proscribes, for protection against thermal neutrons, the use of cadmium screens - emitters of gamma-rays by a reaction (n, gamma) - which give the most distinct cut-off between thermal and epithermal neutrons.
- Ionographic detectors, on the other hand, are completely insensitive to gamma-radiation and can therefore be used under cadmium. Unfortunately, they are rather cumbersome to use, since they require a treatment to reveal latent traces and have to be read by means of various optical or electrical processes none of which is entirely satisfactory at present.

Project two considers the design and construction of a portable environmental dosimeter.

Likewise on the basis of epithermal neutron detection, it is possible to design environment dosimeters which would be lighter than the "rem meters" with a moderator (2 kilograms instead of 10) and simpler than the LET spectrometry systems.

Devices of this type, adapted to spectrometry and beam calibration, are being studied at the Cadarache Laboratory. They were presented at the IAEA Congress held in Stockholm in June 1978. It would be possible to simplify this equipment in order to produce devices for industrial use (light, easy to handle, sturdy and self-contained). The study will be concerned with the use of a uranium-235 fission chamber.

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Contract no.: BIO-A-294-81-F

General subject of the contract:

Theoretical and experimental analysis of the ionizing track pattern in dense tissue-like media.

Description of research work:

The need for reliable knowledge of the various mechanisms ruling the interactions of heavy charged particles (HCP) with biological tissues calls strongly for experimental and theoretical studies in this field. Light and heavy secondary products will be measured along recorded HCP trajectories within wide intervals of the parameters involved. These experimental data, which are urgently required in recent literature, will be interpreted by means of theoretical formalisms which have to be developed or adapted. Together with range-energy determinations, experimental as well as theoretical, the results obtained will lead to a realistic ionizing track structure model in condensed matter.

Since most of the existing theoretical treatments intended to calculate the delta-ray productions along primary HCP are or not rigourous enough in the description of the ionization process, especially as far as the angular distributions are concerned, or lead to quite complicate formalisms resulting in programs too unhandy and too costy for practical purposes, the development of ionization cross sections differential both in ejection angle and energy (D.D.C.S.) has to be continued. The validity of these new treatments will be verified by comparison with measurements carried out along alpha-particles recorded in ionigraphic detectors which reflect the angular distributions as well as the yields of the delta-rays, and with experimental data from the literature. Furthermore, the flexibility of the treatment has also to be verified as far as the extension to other HCP's is concerned. These D.D.C.S. can then be applied in various fields of interest, e.g. as constituent of a track model, or by introduction in expressions of the stopping power, leading to determination of ranges, W values, etc.

It has already been shown that the number of energetic ($E_{recoil} > 1$ keV) recoil nuclei is much higher than generally admitted (of the order of several recoils/5 MeV alpha-track) and they have therefore to be taken into account in the evaluation of the effects of HCP on dense organic matter. These determinations will be continued by means of a somewhat sophisticated methodology, including improved ionographic treatments combined with refined image analysis, in order to obtain accurate, reliable, experimental cross-sections. Starting from working hypotheses based on experimental facts, various theoretical approaches will be attemped, notably by flexible formalisms taking into account interaction cross-sections and stopping powers. The chosen model will have to be verified by comparison with experimental data for a wide

range of projectile and target parameters.
In the evaluation of the damages induced in biological tissues by HCP's, the spatial extension of the interaction volume has to be taken into consideration, which is determined by the range of the primary particle as well as by the pathways of the ejected secondaries, especially the energetic recoils. Different types of visual detectors will be exposed to HCP's of different Z, at various charge states and over wide energy range, and the corresponding pathways measured by means of different image analyzing approaches. These experimental data will be compared with first and higher order moments for the range calculated by means of various approaches based notably on different expressions of the stopping powers related to the interaction processes involved in the slowing-down of a HCP.
In order to determine the spatial distribution of the stochastic energy deposition events surronding the trajectory of a HCP traversing a tissue, a model of the ionizing track will be proposed based on the interaction processes and track parameters mentioned before. This simulated pattern will be compared with the materialized structure. After testing, the model will be applied to the description of the track pattern in the vicinity of the primary's trajectory where measurements are practically no more feasible. Along the same line, the linear structure of materialized tracks will be analyzed. An interpretation of eventual systematic variations will be attempted in terms of ionization, charge exchange, etc. Finally, the relatively high occurence of arborescences, i.e. of successive energetic nuclear collisions, will be simulated and introduced together with the corresponding end of trajectory cascades in the track model proposed.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-A-309-81-UK

General subject of the contract:

Solid state physics underlying the properties of luminophors used in luminescence dosimetry.

Description of research work:

The overall aims of this programme are (1) to gain a better understanding of the fundamental processes involved in luminescence dosimetry, (2) to apply luminescence dosimetry techniques to personal (individual) monitoring, clinical, environmental, mixed field, charged particle, and surface dosimetry, and (3) to identify new areas of application.

Specific areas of investigation will include the following :

Dosemeters for specific applications

High sensitivity phosphorus have been, and are currently being assessed for personal, clinical and environmental dose measurements. These include new high sensitivity forms of lithium fluoride containing copper and phosphorus ; magnesium borate containing thulium and dysprosium ; lithium borate containing copper ; calcium sulphate containing thulium and dysprosium ; and calcium sulphate containing dysprosium (TLD 900).

Photo-transferred thermoluminescence (PTTL) and reassessment of absorbed dose

The NRPB uses the PTTL technique routinely for the reassessment of absorbed dose on insert dosemeters in the NRPB national TLD service. An optimum UV-thermal irradiation regime has been developed. A semi-automated UV exposure unit is presently under construction. A current line of investigation which will be studied further is the effect of multiple reassessment irradiations on the relative stability of the reservoir of deep traps in LiF:Mg:Ti. It is hoped that information about the mechanism of PTTL and the nature of the deep traps will be obtained from these studies.

To contribute to all of these studies, a versatile TLD reader assembly is being built, enabling irradiation with alpha, beta, gamma, X and UV radiations over the range of temperatures -200 to +300°C. This will be used for the examination of the glow curve structure of TL phosphors (in particular LiF:Mg:Ti). Initially areas of interest will include (1) the behaviour of glow peaks with various annealing, handling and cleaning procedures, (2) the dependence of relative glow peak heights on the quality (LET) of the radiation and (3) the growth and decay characteristics of glow peaks associated with PTTL.

Lyoluminescence studies

It is intended to continue this work and investiage (1) the feasability of using a combination of a lyoluminescent and thermoluminescent

detector as a criticality dosemeter and (2) the feasibility of using lyoluminscent detectors (mannose or glucose monohydrate) for radiotherapy dose measurement - in particular for neutron and Pi- meson beams. There is great scope for the improvement of the sensitivity of lyoluminscent materials by (1) optimising annealing, (2) improving instrumentation, (3) using sensitizers. As with TLDs, the dependence of response on LET is important and will be investigated further.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-291-81-D

General subject of the contract:

Development, investigation and application of a reference beams of monoenergetic neutrons.

Description of research work:

Thermal and intermediate neutrons and high-energy photons (E >3 MeV) play a major role in personal dosimetry and workplace monitoring through the dose equivalent rate to which they give rise. In order to apply measurement procedures to them, appropriate calibration fields are required. For these purposes, radiation tubes at the Braunschweig Research and Measurement reactor (Forschungs- und Messreaktor Braunschweig (FMRB)) of the Physikalisch-Technische Bundesanstalt (PTB) are to be equipped with irradiation facilities.
The FMRB irradiation facilities are to be adapted as calibration stations and will be made available to interested bodies from the entire European Community area.
In order to achieve this objective, the following work will be necessary:

- determination of radiation field parameters (spectral neutron flux density and its spatial distribution, photon radiation);
- reduction of background high- and low-energy neutron components, photons);
- determination of the dose-equivalent components with the aid of known conversion factors;
- establishment of a suitable calibration procedure for radiation protection measurement devices (minicomputer-controlled), to facilitate routine measurements;
- development of suitable monitor systems for the monitoring of radiation properties;
- development and construction of a radiation tube plug-in device to produce a beam of high energy gamma radiation;
- development of a spectrometer system to determine radiation field parameters by which the photon dose equivalent rate can be measured.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-308-81-UK

General subject of the contract:

The development of a rationalized approach to the dosimetry of external beta and photon radiation for radiological protection purposes.

Description of research work:

The objective of the study is to enable radiation protection measurements of external radiation, particularly radiation survey measurements of beta and photon radiation, to be related as directly as possible to the dose equivalent limits specified by the ICRP. These ICRP limits are specified in terms of the quantities effective dose equivalent and dose equivalent index.

Since the quantity dose equivalent index cannot be measured in most practical situations, other, more practical, dose equivalent quantities require to be specified and determined.

A programme of work to determine experimentally the relationships between certain of these quantities and the quantities realized by existing radiation standards such as exposure and absorbed dose to air, which have traditionally been used for radiation protection measurements, particularly for measurements by operational survey instruments has been concluded.

The data obtained from these measurements will be used for the formulation of criteria for the design of measurement devices to indicate directly in terms of these new quantities and the development of practical radiation detectors to meet these criteria. The quantities under consideration are all specified in terms of the doses in a phantom of some kind, usually a 30 cm diameter tissue equivalent sphere. In any given radiation field the dose in the phantom is largely determined by the attenuation and scattering of the radiation within the phantom. For area monitoring, however, any measuring device will be used in free air. The particular problems to be studied are the means by which a detector in free air can be made to have the appropriate response characteristics in terms of the energy and angular distribution of the incident radiation to indicate the "in-phantom" quantity.

All measuring instruments used for radiation protection purposes require calibration and it is intended to study the feasibility of producing dosimetric standards designed to act as calibration standards for the new quantities that can be made directly traceable to existing metrological standards.

In the case of photon radiation, now that it has been agreed that therapy level dosimetric standards should be in terms of the absorbed dose at specified depths in a specified water phantom, it would be desirable to study the relationship between such standards and the

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RADIATION PROTECTION PROGRAMME

Continuation contract no.: BIO-A-308-81-UK

proposed protection level standard in the hope that both therapy level and protection level calibration standards can once again be linked to primary standards by a common metrological chain.
In the context of beta radiation it is intended to study the angular distribution of the contributions to the beta dose rate both in air and near the surface of a tissue equivalent material with the eventual aim of developing a measuring device suitable for field use, to determine the dose to skin as specified by the ICRP.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
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Contract no.: BIO-A-311-81-NL

General subject of the contract:
Collection and evaluation of neutron dosimetry data.

Description of research work:
The future CENDOS activities will be concentrated on the collection and evaluation of data relevant for the quantitative assessment of neutron effects in biological material. In the area of neutron dosimetry for radiation protection, the activities on the acquisition of basic physical parameters and characteristics of instrumentation should be identified. Adequate neutron dosimetry methods, both experimental and theoretical, are necessary for the interpretation of biological results and for the evaluation of risks from neutron exposure. In general the 1981-1984 CENDOS program will include the following activities :

- collection and evaluation of data, useful to harmonize neutron dosimetry for beams and radiation protection fields and to a better understanding of biological effects.
- sponsoring of intercomparisons and calibration efforts.
- stimulation of small projects on data acquisition.
- provision of tools necessary to achieve the above mentioned aims including preparation of data tables, computer programs, prototype and/or standard type dosimeters and related instrumentation.
- reviewing of the current status of the field through the organisation of meetings, workshops, seminars, etc.

Within this framework four different research areas can be distinguished :

a) The interpretation of the reading of a dosimeter in a mixed field of neutrons and photons, with wide spectral distributions, in terms of absorbed dose, requires knowledge of a number of basic physical data including cross sections, kerma values, stopping power data, W values and neutron sensitivity of the photon dosimeter in dependence on the neutron energy. For a better understanding of the effectiveness of high LET particles, information should further be collected on track structure, particle fluence spectra and other basic quantities correlated with quality factors, etc.

b) In neutron radiation monitoring special cooperative effort will be placed on the development of new personal dosimeters (e.g. the albedo and track etch detectors), development of new area dosimeters (e.g. light weight rem-counters or dose equivalent index counters), compilation of parameters characterizing the radiation protection fields (e.g. by the multisphere method) and research on the determination of conversion functions for index quantities and effective dose equivalent by means of numerical and experimental

methods. Calibration and practical measurement methodology for area and personal monitoring under routine, operational and accidental conditions.

c) Coordinated programs of neutron dosimetry intercomparisons have shown the existence of large discrepancies between the values of absorbed dose determined by different participating groups. The discrepancies had to be attributed to the use of different basic physical parameters and to systematic differences in measurement procedures. In order to check whether dosimetry procedures and experimental arrangements have been improved, a limited number of on-site dosimetry intercomparisons will be performed at the neutron fields actually employed at institutes working on radiation protection and neutron radiobiology. In the area of neutron monitoring instrumentation special attention should be given to the calibration of instruments.

d) The evaluation and assessment of occupational neutron exposure by systematic inquiries, working place analyses, field studies and results of personal dosimetry. In cooperation with other bodies, these results should be used for further re-estimation of neutron radiation risks.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-A-312-81-US

General subject of the contract:

Quantities, units and measurement techniques for ionizing radiation.

Description of research work:

The International Commission on Radiation Units and Measurements (ICRU), since its inception in 1925, has had as its principal objective the development of internationally acceptable recommendations regarding :

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

The ICRU also considers and makes recommendations in the field of radiation protection. In this connection, its work is carried out in close cooperation with the International Commission on Radiobiological Protection (ICRP).

The ICRU endeavors to collect and evaluate the latest data and information pertinent ot the problems of radiation measurement and dosimetry and to recommend the most acceptable values for current use. The Commission's recommendations are kept under continual review in order to keep abreast of the rapidly expanding uses of radiation.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-339-81-DK

General subject of the contract:

Radioecological studies in temperate and arctic waters of the North Atlantic region with emphasis on transuranic elements.

Description of research work:

Different sources of radioactive contaminations of the North Atlantic region have been studied for several years by the Health Physics Department at Risø. As a whole the fallout nuclides from nuclear weapons testing are still the main source of man-made radioactivity in the North Atlantic region. However in local waters other sources may prevail. In the North Sea most of the radiocesium thus comes from reprocessing plants for nuclear fuel in Western Europe. In the inner Danish waters a reactor-produced corrosion product such as Co-60 is easily measurable, and at Thule, Greenland a local contamination with Pu and Am has increased the levels of transuranics. It is the intention to continue these studies in the eighties. Special attention will be paid to intercomparisons among the activity levels found in seawater, sediments, sea-plants and mussels related to salinity and temperature. The environmental studies will furthermore be supplemented by experimental studies.

1. Experimental studies. Mussels (Mytilus) and benthic brown algae (Fucus) are widely used as "bioindicators" for the distribution of radioactive as well as conventional pollution in coastal marine and estuarine environments. The main aim of this research programme is to quantify the effects of important environmental parameters on rate of accumulation and loss of certain transuranic-, fission- and activation-nuclides, and consequently to evaluate and improve the use of these organisms as bioindicators for the distribution of long-term contamination, e.g. fallout, controlled discharges from the nuclear industry and uncontrolled releases after accidents. During the last 3 years equipment for culturing mussels under controlled but natural food conditions has been developed. The sophisticated set-up is able to keep a constant but very low phytoplankton suspension in the water giving the mussels natural food conditions. Experiments with transuranics (Pu, Am, Np, Cm), but also rare earths, e.g. Ce and Eu will be started. After accumulation in the laboratory the excretion will be studied in the field. The fucoid brown algae are even more efficient concentrators of these nuclides than are Mytilus. It is the intention to study the uptake and loss of relevant radionuclides, especially Pu, Am, Ce, Eu, Cs, Co, Zn and Mn by Fucus in the laboratory under different environmental conditions. For both mussel and algae studies it is, if possible, the intention to study the uptake of different relevant chemical forms and to measure the particulate

activity during the uptake in order to evaluate uptake via phytoplankton and other particulate sources. Special attention will be paid to the effect of low temperatures in order to make the data comparable to conditions in Greenland and in Northern Europe in winter.
2. Environmental studies. The contamination with transuranics, radiocesium, tritium and cobalt-60 of the North Atlantic Region will be studied in marine samples of seawater, sediments, seaplants and mussels. The seawater will be collected annually from a number of stations in Greenland, the Faroe Islands and Denmark, which have been sampled during the last two decades. Using the new Danish Oceanographic Research vessel DANA, deep water as well as surface water samples will be collected. The field methods developed at the Thule 1979 expedition to concentrate radionuclides from large (1700 l) seawater volumes will be applied. By double-tracer technique (Pu-236 and Pu-242) the oxidation steps of Pu in seawater will be identified. Particulate activity will also be measured. In 1980 seawater samples will be collected in arctic waters by a Swedish scientific polar expedition to Spitzbergen. These samples will be analysed in cooperation with Risø in 1981. Sediments will be collected from the North Sea and if possible in the North Atlantic Ocean between the Faroe Islands and Greenland. Special attention will be paid to the analysis of transuranics and radiocesium in order to identify any accumulation in sediments of radioactivity released from reprocessing plants. Seaplants (Fucus, Laminaria, Ascophyllum) are sensitive bioindicators of many radionuclides in seawaters. Concentration factors of transuranics are in the order of 10^3-10^4 depending upon radionuclide, algae specic and environmental conditions (e.g. salinity). The releases from reprocessing plants in Western Europe pass northward along the Norwegian West Coast. A preliminary study of radionuclides in seaplants will be carried out in Norway in 1980. Samples from this study will be analysed at Risø. Similar studies are planned at Spitzbergen (Sweden) and along the East Greenlandic coast (Denmark). Mussels (Mytilus edulis) will be collected in Greenlandic, Faroese and Danish waters and compared with fucoids where both are available in order to evaluate their relative ability as bioindicators for radioactive contamination in various environments.
3. Thule studies. Samples collected at previous expeditions (1968, 1970, 1974) will be analysed for Am-241, as this analysis has now been developed. Old Pu-counting samples from the in-growth of Pu-241. In 1984 a fifth expedition to Thule is planned. At this new expedition results from the earlier samplings will be followed up and the results obtained from the laboratory experiments with transuranics will be as far as possible be included in the investigations.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-B-439-81-UK

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General subject of the contract:

Radionuclide pollutants in natural plant-soil ecosystems.

Description of research work:

The research programme is concerned with the distribution and movement of radionuclides within the natural terrestrial ecosystems. At present most of the interest is centred on the fate of these nuclides when deposited on vegetation and surrounding soils. Experiments have been established to look into :

1. The amount of radionuclides taken up by contrasting plant types.
2. The relative importance of root and foliar uptake of the nuclides.
3. The importance of root and foliar morphology and of leaf texture in determining the tension and absorption of the nuclides.
4. The distribution of absorbed radionuclides within different plant components.

Corresponding collections and measurements of field materials will also be carried out and the results assessed in relation to the experimental data and environmental variables.

The importance of grazing on contaminated pastures (especially by sheep) as a pathway to man is being examined. Food, fecal droppings and sheep carcass samples are being analysed in the course of this work. Other factors which influence the uptake of radionuclides by grazing animals, such as management practice, behaviour, gestation and age are being taken into account.

Studies on the variation of radionuclide levels in the vegetation and sediments of ungrazed and grazed saltmarshes have been completed and the results are being prepared for publication.

Most of the field work is being carried out in an area within about 30 kilometers of Windscale Nuclear Fuel Reprocessing Plant in West Cumbria, United Kingdom, since the levels of pollutant radionuclides are generally higher in this area than elsewhere in the country. This is a very suitable area for ecological studies because it contains contrasting types of vegetation, topography and land use all found in association with a range of different soil types. This area is also very convenient to the base laboratory at Merlewood Research Station where the experimental and analytical tests are being carried out.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-465-81-F

General subject of the contract:

Development of a method of determining trace of long-lived beta-emitters by opto-galvanic spectroscopy and assisted fluorescence.

Description of research work:

Measurement by counting techniques of very low concentrations of long-lived beta emitter isotopes such as technetium, iodine and tin is fraught with genuine difficulties. Any study of transfer in the environment necessarily involves a stage comprising the determination of their contents in the compartments concerned : water, soil and biological tissues. The reliability of the results is closely bound up with the quality of the values obtained from the analysis of the elements; quite apart from this reliability, it is indispensable that the analytical technique should produce the results quickly and at a reasonable cost.

It is quite evident that the lack of information concerning iodine-129 and plutonium is connected with the difficulties encountered during the measurement. The toxicity of these beta emitters has prompted and will prompt more and more in-depth studies of their behaviour and, consequently of their measurement. Owing to the low energy of the emitted radiations, the methods based on the use of such emission will always be difficult to employ. It is of course, possible to use artefacts, such as iodine-129, in-pile irradiation and analysis based on the iodine-130 isotope formed. These techniques, although they have now been perfected, are time-consuming, since they usually necessitate in addition to irradiation chemical separation, prior to counting and they do not provide a possibility for continuous survey.

In order to overcome these disadvantages it is proposed to apply to the analysis of these elements an optical spectrometry method suggested a few years ago by research workers of the National Bureau of Standards : opto-galvanic spectroscopy (laser enhanced ionization). This method consists in specifically exciting by laser radiation element at a level close to its ionization potential; a specific ionization will result and the quantity of ions produced can be measured with an ion collector. It may be pointed out that the same device with an optical detector measures the concentrations by means of optical fluorescence. Both techniques are very sensitive, the sensitivity in each case being dependent upon the element analysed. The published values on laser-excited fluorescence are more numerous than those obtained by the opto-galvanic effect; nevertheless, limits of detection of the order of one picogram were recently reported in the case of cetain elements such as sodium in water.

Our laboratory has carried out a series of opto-galvanic spectroscopy

measurements with flame atomization that corroborate the obtained results.
We therefore intend to apply these techniques to the determination of long-lived beta emitters. The lack of spectral data on some of these elements will necessitate basic research prior to the method of determination. The working programme will mainly focus on the measurement of iodine and only later on the measurement of technetium.
For the determination of iodine, two possibilities will be investigated, namely opto-galvanic spectroscopy and fluorescence. In the former case, a theoretical selection of the spectrum-lines specific for I_2 - 129 and most sensitive for an excitation up to 1 or 2 photons will be done. Thereafter, the most suited spectrum-lines with regard to sensitivity and reproducibility, will be studied experimentally and the opto-galvanic spectroscopy will be adapted. A circulation measurement cell with an arc-type unloading will be studied for gaseous measurements. Attention will be paid to the influence of compounds eventually interfering with the measurement of I_2. For the measurement by fluorescence, a similar programme will be executed. The final stage of the procedure will consist out of the measurement of air and gas samples after dissolution in order to test the real performances of this method.
For the determination of technetium, only the opto-galvanic spectroscopy seems to be usable. The work will be limited to the adjustment of a closage method in aqueous media by flame atomization.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-340-81-F

General subject of the contract:

Simulation of tritium transfer in the environment; study of organic tritium transfer in the aquatic environment, the soil-plant and the plant-mammal system.

Description of research work:

The proposed study contains four research projects intended to provide maximum comprehension of the tritium cycle in the environment. Given the complexity of the tritium cycle, the most rational approach likely to lead to an overall understanding rather than partial incomplete knowledge is to combine both modelling and experimentation. This approach assumes that the model is initially at a slightly more advanced stage than the experimental work and that a continual feedback between research workers engaged in designing models and those engaged in field and laboratory experiment is set up. The four interrelated projects involve the modelling of the behaviour of tritium and an experimental study of the transfer of organic tritium in the aquatic environment, the soil/plant system and the plant/mammal system. These projects might possibly be completed by contributions from teams working on the conversion into organic form of tritium in the soil and on tritium exchanges between the soil and the atmosphere.

1. Modelling of the behaviour of tritium. The ultimate aim of this work is to establish a general model of the behaviour of tritium in the environment as a whole. This will mean that attention must first be focussed on subsystems such as the aquatic environment, soil/plant relations and plant/mammal relations (including animal metabolism). These subsystems will then be combined in a larger model incorporating tritium transfer in the atmosphere, rivers, estuaries and oceans. An approach of this kind was used by NRPB/CEA to describe the behaviour of a large number of radionuclides, but the particular aspects of tritium make this model totally unsuitable and mean that, for tritium, the approach to modelling and experimental proof must be along different lines from that used for the other radionuclides.
2. Behaviour of tritium in different chemical forms in the freshwater environment. Description of this research programme is given under contract number BIO-B-431-81-B.
3. Behaviour of tritium in the soil-plant system. Description of this research programme is given under contract number BIO-B-431-81-B
4. Behaviour of tritium in mammals. Description of this research programme is given under contract number BIO-B-432-81-NL.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-324-81-I

General subject of the contract:

Characterization of particulate contamination of the air and its deposition in the airways.

Description of research work:

For a more realistic dosimetry of radioactive nuclides in the airways, and in the body in general, a more detailed knowledge of the transfer efficiency from the air to the body is needed. The deposition (intended as deposition efficiency, both total and regional) of the particulate contamination depends strongly on particle size distribution.

At present the deposition data for ultrafine particles (i.e. the size range when diffusion mechanisms prevail) is scarse. In addition the role of electric charges deposited on the particles in the complete size range, has been assessed in the case total deposition, and a definite increase has been found; nevertheless their influence on the regional distribution of the deposited material needs to be investigated since if could play an important role in deposition and perhaps in possible hot spots. This is particularly important for alpha active particles.

Therefore it is considered worthwhile studying these problems of deposition, as well as characterizing in more detail the alpha potential energy distribution over the entire size range of interest to airways deposition.

- Deposition measurements of ultrafine aerosols on volunteers. This comprehens the set-up of a narrow distributed silver aerosol generator, of the characterization techniques by diffusion and electron microscopy. The aerosol will then be administered to volunteers by means of the exposure equipment already used at the laboratory for particles of larger sizes (d > 0.3) modified to house a condensation nuclei counter that will be used to measure the inhalation and exhalation concentrations.
- Regional deposition of charged particles as compared with neutral particles in the size range above 0.2 μm.

The laboratory has the capability of producing radioactively labeled monodisperse aerols : their deposition, clearance and leaching has been already studied under a previous contract in rats. They will be charged with a narrow charge number distribution in a corona discharge apparatus, already used at the laboratory. This aerosol will be administered to volunteers and the regional distribution will be assessed by means of a whole body counter that will be calibrated for lung measurements. This distribution will be compared with the distribution obtained in equal conditions with equal aerosols without

electric charge. This is considered important since deposition enhancement is by image forces i.e. it is due to the charge individually carried by each aerosol particle. Aerosols freshly generated by mechanical disruption (nebulisation, grinding blasting) are frequently encountered : they carry a large average absolute charge and their deposition is greatly enhanced. This research should offer guidance to establish if and to what extent particle charge should be characterized in pratical situations.

- As for alpha emitting aerosols a more detailed size distribution is needed both in the aerodynamic range and in the diffusion range.

In the aerodynamic range, encouraging preliminary results have been obtained in combination of a newly developed inertial spectrometer and a CR 39 track detector for the determination of potential alpha energy distribution as a function of size even for short lives. A working field spectrometer should be developed to measure the size distribution of particles containing radon and thoron daughters.

This should be complemented with a research aimed at characterizing ultrafine particles with diffusion techniques; we think it appropriate that size characterisation should be performed by means of the mechanisms that are actually active in deposition.

This will be done by means of a diffusion battery in a rectangular geometry that will contain track detectors at various distances; this will give indication on potential energy concentration as a function of the residence time and hence on the effective diffusion coefficient. These two will be combined in one instrument in order to characterize the alpha active aerosol in the complete size range of interest to inhalation toxicology.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-466-81-F

General subject of the contract:

Resuspension of radioactive nuclides released from the ocean surface.

Description of research work:

1. Laboratory study of resuspension of radioactive nuclides released from the ocean surface

The Atmospheric Transfers Laboratory will use a laboratory-scale device by means of which it will be able to produce bubbles in sea-water sampled close to the shore and collect the aerosols formed in order to study their size and composition. By means of this experimental device it will be possible to vary some parameters (bubbling, composition of sea-water, physico-chemical form of radioactive nuclides, etc.) and to study the effect of such variations on the resuspension phenomenon. Real conditions will be imperfectly reproduced but, on the other hand, it will be possible to analyse experimentally the basic phenomenon, which is much more difficult in experiments performed in situ. The sea-water used in each experiment will be analysed in order to determine the organic matter content. The radioactive nuclides will be inserted in the form of a cocktail of fission products and gamma-emitter actinides (Pu-237, Am-241).

Sodium-22 will be added in order to trace the stable sodium. The aerosols formed will be collected on a impactor with several stages so as to fractionate the particles according to size. The radioactivity deposited on each stage of the impactor will be measured by high-resolution gamma spectrometry. It will thus be possible to determine, in the case of each granulometric fraction and each radioactive nuclide, an enrichment factor which may be expressed in conventional form as

$$F = \frac{(M/Na \text{ aerosol})}{(M/Na \text{ sea-water})} - 1$$

where : M = radioactive nuclide counting rate and Na = sodium-22 counting rate. In these laboratory experiments care will be taken to use radioactive nuclides that are in a well defined physico-chemical form which has been determined resewhere.

In the case of the actinides in particular a careful distinction will be made between the hydrolysed forms and the complexed forms, the proportions of which will be varied, allowance being made for the process of sea-water contamination. Special attention will also be paid

to the organic matter entrained by the aerosols. The expectation is that as a result of these experiments the phenomenon of resuspension of radionuclides released from the micro-layer will be elucidated and that the enrichment factors will be assessed.

2. In situ study of resuspension of radionuclides released from the ocean surface

In an intial stage, the Atmospheric Transfers Laboratory will develop high flow-rate sampling devices. The finding from its preliminary experiments is that a 500-600 m3/h sampling flow would be suitable. These devices will effect granulometric separation so that it will be possible to distinguish the respirable fraction from the non-respirable fraction with a cut-off diameter of approximately 5 µm. As far as possible, the laboratory will endeavour to work in situ and to obtain approximately isokinetic sampling by adjusting the suction orifices so that the inlet-flow speed is close to wind speed. Generating sets of suitable output, will be used in order to be able to select the sampling sites without constraints of electric power supply.

In order to carry out the necessary comparisons, three sites will be studied : (1) a coastal site open to gusts of wind from SW to NW located very near to the discharge point of the La Hague reprocessing Ecalgrain Bay plant. (2) a coastal site similar in outline, but located a long way from any industrial discharge (to be specified); (3) an inland site in the Paris region for example.

Comparative samples will be taken simultaneously at the three sites for periods of a week to a fortnight during which weather conditions, and more especially mean wind speeds, differ. In the course of each period and at each site it will be necessary to filter approximately 30.000 m3 of air corresponding to the volume found to be necessary during our preliminary tests.

The fission or activation products will be determined on filters by means of high-resolution gamma spectrometry. The actinides, and first of all plutonium and americium, will be determined by the convetional radiochemical methods. Sodium and any organic matter will be determined by the usual processes.

During each series of operations a sea-water sample will be taken at each coastal site. The volume of these samples will be some hundreds of litres. Using these samples, radioactive nuclides will be determined, if possible by separating the particulate colloid and soluble forms. This work will be done by the Marine Radioecology Laboratory at La Hague.

By comparing the results obtained, it will be possible to assertain resuspension of nuclides released from the ocean surface significantly increases the concentration of certain nuclides in the air at least locally. The data collected in situ will be compared with those collected in the laboratory.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-440-81-UK

General subject of the contract:

Behaviour of tritiated water in trees and other vegetation.

Description of research work:

This programme forms part of a continuing study of the behaviour of tritium in the environment. The purpose of the study is to provide the information required for the estimation of the radiological consequence of the release of tritium or tritiated water from nuclear plant. To this end the conclusions of this work will be used in the development of the Tritium Behaviour Model by Dr. Gerber at CEN/Mol.

This present programme concerns the behaviour of tritiated water in trees; and other large plants. Despite a number of useful investigations into the behaviour of tritiated water in vegetation, the influence of environmental factors on the concentration of HTO in various parts of a plant has not been quantified. It is generally assumed that HTO is transported through the soil-plant system by the flow of water, but recent work has shown that diffusion through the soil water can sometimes be important. In plants, diffusion and vapour exchange can make the tritium concentration in leaf water many times less than the concentration in soil water. Simple physical reasoning suggest that a plant should contain:

a) "Vascular" water confined within water vessels in the stem and not susceptible to exchange with atmospheric water, and
b) "leaf water" derived from vascular water by exchange with atmospheric water.

A simple relationship is expected to exist between the HTO concentration in leaf water and that in the vascular water, according to the transpiration rate, and stomatal resistance. Confirmation of this relationship would allow prediction of the behaviour of HTO in a wide range of crops, and the exchange of HTO between tall crops and the atmosphere. The residence time of HTO in plants with a substantial volume (such as trees) may be important in determining the time period during which precautions are necessary following an accidental release of activity from a reactor or other nuclear installation.

It is proposed to investigate the relationship between HTO concentrations in various parts of a plant and monitor the transpiration rate and resistance for exchange of atmospheric water simultaneously. Initially small trees form suitable subjects since

a) HTO may be injected into the stem in known amounts.
b) it is easy to distinguish green leaf tissue from woody tissue

which contains vascular water only.

c) the size of the plant provides ample material for sequential samples and facilitates transpiration measurement in a single individual.

Transpiration will be measured by enclosing in a plastic tent and measuring the change in humidity of a known flow of air through the enclosure. In addition, the residence time of the activity in the tree will be measured and related to the rate of transpiration, and the size of the tree. Similar methods would later be developed for food plants including leaf, root and grain crops, so that the relationship between the concentration in the edible parts and the water supply could be investigated.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-322-81-I

General subject of the contract:

Environmental and health protection implications from nuclear plants discharging into coastal marine ecosystems.

Description of research work:

The research programme is essentially related to the need to improve predictive capability for longer-lived radionuclides, whose behaviour now needs to be considered over much longer time scales in order to give effect to the new ICRP emphasis on estimation of collective dose equivalent commitment as part of the need for optimization. The central problems therefore surround the need to examine those physical and chemical factors which determine the initial fractionation of activity and its subsequent transport and distribution within the marine environment, especially between water sediment and suspended matter as these compartments are the prime determinants of any initial distribution, but also the way in which these compartments act as reservoirs for uptake into food chains leading to man.

To implement the above mentioned goals both environmental studies and laboratory investigations are required. The results of the studies on existing nuclear plants will be used to understand the mechanisms of distribution of the radionuclides in the different compartments of the marine environment. In particular the chemical, biological an physical parameters governing the distribution of radioactive and/or stable elements between water, suspended matter, biota and sediments will be considered. Samples will be collected according to a scheme based on a geomorphological classification of the Italian coasts. Physical oceanographic research devoted to the study of turbulent mixing and transport will be carried out to develop suitable models describing the dispersion patterns of radionuclides released into the marine environment.

Chemical, mineralogical, biological and physical data will be used in an integrated way to describe and, where possible, quantify the phenomena governing the distribution into the marine environment of radioactivity due to the operation of nuclear installations. In particular the chemical composition of sea sediments (from the point of view both of macro and micro constituents) will be determined.

Studies on oxidizable organic matter will also be made due to the importance of this material in controlling (with the degree of O_2 saturation) the redox conditions of the sediments which in turn play a major role in the mobility of elements whose solubilization depends on the oxidation state (e.g., Pu, Np, Tc, Fe, Mn, Cr, etc.) In this context the determination of radioactive and/or stable elements in pore waters is of great importance because it would permit to evaluate

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possible fluxes of pollutants from sediments to water or, vice versa, the diffusion of pollutants into deeper sediment layers. Laboratory studies aimed at determining the distribution coefficients between sediment water and sediments for some radionuclides will be performed; the effects of bacteria and humic matter on the distribution coefficients will be investigated with the scope of contributing to the assessment of the effects of those poorly known factors on sorption of elements by sediments. The behaviour of different chemical forms of long-lived radionuclides will be also studied by means of experiments under controlled conditions.The low level measurements of natural and fallout radionuclides will be carried on in a few sampling stations along the Italian coasts. The results of this work together with data obtainable from studies performed in existing nuclear sites will help to provide advise and guidance on the need for, and scale and character of, radiological impact assessment for operating nuclear installations discharging radioactivity to the marine environment or in the context of accident situations. The laboratory will also participate into the research program organized by NEA and devoted to the control of ocean disposal of low level radioactive waste. As a contribution to the study of natural alpha- emitters, Ra and Po will be determined in water, sediments and biota and the food chain "water-fish-squid-man" will be studied. Studies shall be initiated on the behaviour of transuranic elements by both laboratory and field investigations. The results obtained on the different projects of this proposal will be used to determine the dose equivalent to critical groups and the collective dose equivalent commitment in the areas studied and referring to different release conditions of real or hypotetical situations. For this purpose both already available and new ad hoc models will be prepared and applied.

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Head(s) of research teams(s):

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Contract no.: BIO-B-323-81-I

General subject of the contract:

Biological and environmental aspects of toxicology of plutonium.

Description of research work:

The risk to man due to plutonium and other actinides has been the subject of increasing debate in recent years. This debate has had a significant impact on public opinion which has been actually lead to believe that "plutonium is the most dangerous substance now available to man".

Beside this public concern further data are also needed in the field of toxicology of plutonium with particular reference to :

a) Dose-effect relationships in various animal species at low dose.
b) Dose calculation in various organs and tissues of both animal and man.
c) Fallout plutonium levels in diets and in members of the general public.
d) The levels and behaviour of fallout plutonium in soils and marine and continental sediments; transfer from soil to food crops.

Therefore an extensive research program divided in three projects, has been designed to partially cover the above reported items. The first project actually under way is designed to give the dose-effect relationship in mice for bone carcinogenesis induced by plutonium. To that purpose more than 2000 animals have been intravenously injected with monomeric Pu-239 at six dose levels ranging from 30 pCi to 1500 pCi per mouse. All dead animals are subjected to complete X-ray diagnosis, autopsy, histopathology and plutonium determination. A follow-up of a control group is under way at the same time. The late somatic effects of plutonium will be related to the dose evaluation based on the results of the microdistribution studies together with the long-term retention. Together with the main long-term experiment other short-term studies are planned on the following subjects :

a) Microdistribution of plutonium in various tissues (mainly bone and gonads) at various time post injection and as a function of the age at injection.
b) Effects of selected decorporation treatments at various ages and as a function of the chelating agent.
c) Plutonium transfer from mother to foetus as a function of the physico-chemical form of the injected solution.

Furthermore a study in mice of the genetic effects induced by low levels internal contamination of plutonium is also planned by means of a technique based on the evaluation of the chromosome damage in both germinal cells and somatic cells taken from bone marrow and

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spleen. The possible long-term genetic effects will be related to the microdistribution and dose calculation for plutonium content in the gonads and other organs at various times post injection and at various levels of the injected amount.
The fallout levels of plutonium in the environment, in foodstuffs and in members of the general public should be adequately known if the consequences of exposures of the general public to actinides resulting from the nuclear power programme are to be predicted. Therefore a research project has been designed to measure the fallout plutonium levels in diet samples and in various food items, representative of the italian dietary habits and collected in various parts of Italy. The possible existence of critical foods will be examined with particular reference to the measurement of plutonium concentration in sea foods collected in the mediterranean area. Autopsy samples from members of the general public will also be measured to study the actual distribution of plutonium in the human body of the italian population as a function of age. The development of adequate radiochemical methods will be continued to overcome the serious difficulties based on the measurement of the very low plutonium content in these samples.
The third research project has been designed to study the fallout plutonium levels in the main types of soil and marine and continental sediment present in the italian region. The soil samples will be collected in both undisturbed and disturbed sites to show the influence of the human activities on the plutonium behaviour in soil. The plutonium depth distribution in soil will be estimated together with its mobility in the examined soil samples. The influence of various physico-chemical parameters (surface exchange capacity, porosity, pH etc.) of the soils on the plutonium behaviour will be studied. As an end point of this project realistic predictions concerning persistence and dispersion of fallout plutonium in soil should be made as a function of the characteristics of the soil. Furthermore the analysis of fallout plutonium content in the most important food crops will be conducted to study the relationships, if any, existing between the fallout plutonium contents in soil and those in food crops cultivated on the same soil. Continental sediment samples will be collected with the aim to be representative at large of some peculiar deposition situations of Italy. Plutonium content and depth profile will be studied in these samples. The part of the project related to the measurement of plutonium content in marine sediment is partially covered by the research program presented by the Laboratory for the Study of the Marine Environment. The marine and continental sediments and soil plutonium data will be therefore used to have valuable information on the fallout plutonium distribution in the italian environment and to evaluate the consequences of possible releases of plutonium under other physico-chemical forms.

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Head(s) of research teams(s):

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Dr. M.J. Frissel*
Dr. J.M. Martin**

Contract no.: BIO-B-326-81-NL

General subject of the contract:

Differential migration of plutonium in the delta estuaries of Rhine, Meuse and Scheldt.

Description of research work:

Various studies by national and international institutes have demonstrated that sedimentary particles can trap potentially radionuclides. For estuarine systems it is particularly important to know whether accumulation by sediments continues over long periods until maximum concentration beyond which the inputs equal decay and output. This problem is very essential for radionuclides with very long half-lifes like Pu-239. As for stable contaminants, it is necessary to know whether estuaries are infinite sinks or just intermediate pathways for radionuclides. Prediction of these or other processes can be made only on the basis of a proper understanding of (1) the reactions between water and bottom sediments, (2) the transport processes of sedimentary particles loaded with radionuclides including biological mixing.

The Delta Institute for Hydrobiological Research has focussed part of its research on a quantitative interpretation of these processes so that parameters such as residence times in estuaries may be used to give an indication on the expected trends for short and long-term periods. Equally,the ITAL* (Dr. M.J. Frissel, Postbus 48, NL-6700 AA Wageningen) and the Laboratoire de Géologie** (Dr. J.M. Martin, Ecole Normale Supérieure, Rue d'Ulm 46, F-75230 Paris) are involved in related processes on land and estuaries, respectively and have the capacity of analysing Plutonium concentrations in environment samples. The Duursma and Smies" theoretical approaches and related laws are also applicable for plutonium and caesium behaviour in estuaries. The results of previous work demonstrated that certain relationships of plutonium and caesium can be recognised (relation with fine clay fraction) which might forecast a certain behaviour. However, this behaviour has to be investigated in more detail with particular emphasis on sorption to clay particles and on mobilisation as a function of different biotic and a-biotic conditions. The research runs parallel with ongoing studies at the Delta Institute for Hydrobiological Research, Yerseke (general pollution and total alpha-radioactivity), the ITAL-Wageningen (Plutonium measurements on plant and soil samples and physical modelling) and the Laboratoire de Géologie of the Ecole Normale Supérieure of Paris (transuranics, gamma emitters and stable element distribution in rivers, lakes and estuaries). The three institues are studying the differential migration of Pu in estuarine samples as dependent on the possible speciation of

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Plutonium. Their interests are focussed on the migration (1) under oxic and anoxic conditions in waterand sediments (2) in relation to the presence of iron and manganese hydroxides, organic substances (humates) and (3) in dependance of salinity. The programme of the three-partial research concerns : The Delta Institute will sample: particulate matter, sediments, lichens and salt-marsh plants for the analysis of plutonium isotopes (238, 239 + 240), caesium (137), iron, manganese, natural alpha-radioactivity and basic chemical components (organic matter, clay fraction etc.) from which the plutonium and major gamma emitters (Cs-137, Ru-106, Ce-144, Sb-125, Co-60, ...) isotope analysis will be carried out by the ITAL and the Laboratoire de Géologie: the basic chemical components are analysed by the Delta Institute. The group has chosen the sampling points on the basis of points sampled for the 1979-1980 programme and extended to areas not investigated so far. Among them are sampling stations for particulate matter in the mouth of the Western Scheldt and the inshore S.W. North Sea.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-464-81-F

General subject of the contract:

Identification of the nature and the valency states (U-IV, U-VI) of uranium compounds in dusts.

Description of research work:

It is known that inhalation of dusts containing uranium compounds involves considerable hazards; apart from any serious accident, the risk of inhalation is by no means negligible in workshops or mines where large quantities of uranium are handled.

The effects of uranium on the human organism appear to be influenced by its degree of oxidation and, if these relationships are to be conclusively established, quantitative data on the U(IV)/U(VI) ratio in dusts have to be available, while it is also necessary to identify the nature of the compounds : oxides, chlorides or oxychlorides, fluorides or oxyfluorides.

Determinations of the different valency states are usually carried out by electrochemical methods after phosphoric dissolution. A method of this type presupposes that no change is caused by the dissolution; as a matter of fact, it is very difficult, even with thoroughly deoxidated solutions, to prevent partial oxidation of U(IV) into U(VI).

By means of electron spectroscopy (ESCA), valencies can be determined directly on the solid material; the nature of the bond is shown by a characteristic modification of the measured binding.

It is therefore intended to employ this method in order to determine the uranium species in dusts. It should be noted, however, that the presence of peaks called "shake-up peaks" renders the determination more complicated.

The study programme contains two stages :

1. Establishment of basic data
- determination of binding energies of U(IV) and U(VI) for oxides and fluorides. For non-conducting materials like dusts, it is difficult to determine the "real" binding energy. To encompass this, layers of oxides or fluorides on metals will be prepared and the characterization will be done by for instance diffraction of electrons.
- determination of the energies of the "shake-up peaks" by a study of the band structure of the spectrum. These "shake-up peaks" interfere with the primary peaks and the establishment of a computational spectrum analysis programme will be needed in order to estimate quantitatively the valency ratios.

2. Analysis of actual samples from workshops
- dust samples taken from different workshops will be studied, e.g. sintering plants, mines, etc... In order to avoid any perturbation

of energies due to an effect of charge interacting with non-conducting dusts, two procedures may be used :

- redeposition of dusts without segregation upon a suitable sample frame
- collection of dusts upon a metal filter, the metal being sintered, and used directly for spectrometry.

Knowledge of the valency states of the uranium in dusts as a function of their origin will constitute an important information for toxicological studies of atmospheres in working places.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-325-81-NL

General subject of the contract:

Evaluation of long-term behaviour of radiocontaminants - transuranics and activated corrosion and fission products - in different soils and vegetation.

Description of research work:

For a realistic assessment of the health detriment from discharges, reliable parameter values are a prerequisite. An important part of the existing data is not representative for natural and agricultural situations, therefore their use for long-term forecasts and for radiation dose calculations is restricted. This also hampers the elaboration of adequate control and decontamination techniques and procedures. The main objective of the investigation will be therefore the determination of reliable and representative parameter data.

A. Behaviour in soils and uptake by plants of transuranics (Ra included) for different European soil types. A first aspect considered is the assessment of representative data on accumulation and migration in soil and root mediated transfer to plants of Pu and Am for twelve representative soils of the European Community. The transuranics Pu-239 and -240, Pu-238, Am-241 are at the core of the project: Ra-226 is included for reference purposes (and is also an important nuclear waste nuclide as well as a natural radioisotope); U-228, U-235, Th-232 and K-40 are important natural radioactive nuclides which are included because, depending on type of parent material and age of the soil, differences in behaviour are expected; Cs-137 is included for use in model development and Co-60 because of the recent interest in this nuclide. The sampling will be carried out (every two years) on normally grazed permanent pastures. However, not all nuclides
will be measured in all samples. Priority will be given to the most relevant samples and/or nuclides.

A second aspect treated is the study of the migration of Pu in soils as a function of (controlled) environmental conditions. In laboratory studies, the latter (pH, redox potential, organic additives) will be modified so that significant differences in solubility and therefore in migration can be expected. An underlying assumption is that changes in solubility will only be reflected by changes of the solute transport via the waterflux and that particle mediated transport is not modified. This assumption will be investigated by additional experiments.

B. Evaluation of soil-plant transfer of radiocontaminants. The most suitable approach to study under realistic conditions the behaviour of fission and corrosion products (Sr-89 and -90, I-131, Cs-134 and -137, Tc-99, Zn-65, Co-58 and -60, Fe-59 Mn-54, S-35) in the soil plant system is the use of lysimeters that are large enough to grow

agricultural crops without boundary effects. Within the lysimeters, only a limited number of combinations of soils, crops and weather can be considered. Therefore, additional experiments will be carried out in the laboratory to allow extrapolations to other conditions or other crops. The general aim is to determine reliable values for the transfer of radiocontaminants from soil to plant and to evaluate the long term influence of soil physicochemical processes on the fate of the radionuclides. A first aspect will be the determination of the transfer of radiocontaminants for some combinations of soils and crops being representative for important agricultural areas in the European Community. Three soil types, a clay soil, a sandy soil (podsol) and a loess soil (Para braunerde) will be used. In the course of the experiment, regular sampling will be done to determine transport in the soil and changes in availability for plant uptake. The selected crops are crops for human consumption (potatoes, barley, vegetables, beans, peas, tomatoes) and for cattle feeding (maize, grass). An appropriate combination of the following contamination procedures will be applied: a) contamination of the top 5 cm layer to simulate accidental release;b) contamination of the ploughing layer to simulate long lasting continuous releases, c) contamination via contaminated crop residues. Uptake and distribution in various plant parts, e.g. the edible parts, will be measured by Ge-Li gamma-spectrometry and liquid scintillation counting.

A second aspect will be the study of the influence of organic compounds on the availability of various radionuclides, which may be affected by the formation of organometallic complexes. In this respect two processes differing in impact and site of the action can be distinguished: a long-term effect located in the ploughing layer, related to the soil organic matter in this layer and a short-term effect located in the soil-root interphase influenced by the presence of plant produced organic compounds. Both effects may be evaluated by a "double label procedure", i.e. contamination with a second isotope of the same element some years after contamination with the first. The impact of plant produced organic compounds will be studied by growing plants in C-14 labelled carbon dioxide atmospheres and determination of C-14 labelled organometallic complexes.

A third aspect is the soil-plant transfer of Tc. The oxidation potential of the couple TcO_4^-/TcO_2 being 738 mV, TcO_4^- will be reduced in most soils and plants. Leaching of Tc and the presence of reduced forms in soils will be determined. Plant litter from plants that absorbed Tc will be used to simulate contaminations with organic Tc forms. The formation of Tc complexes within the plant causes, at least partially, the sub-toxicity phenomena described in literature. Most of these complexes are expected to be formed with Mn or Mo containing proteins. It will be investigated whether the availability of these metals influences the transfer of Tc.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-B-317-81-F

General subject of the contract:

Behaviour of freshwater entrained radionuclides in the event of contact with seawater.

Description of research work:

The project intends to study the behaviour of the main radionuclides likely to be present in freshwater when this water comes into contact with seawater, bearing in mind the main parameters of the environments involved.

The distribution of radionuclides between water, suspended particulates and sediments can be measured experimentally in the laboratory by a method which defines the distribution coefficient, Kd. This method is a simple way of estimating the distribution between the different compartments : water/solid flow and water/ sediment.

It also takes account of the main parameters :

- nature and physicochemical form of the radionuclides,
- composition of freshwater and seawater,
- nature of solid flow and sediments,
- contact time between the media.

The radionuclides to be studied during the first two years are caesium-137, cobalt-60, manganese-54, iron-54, sodium-22 and zinc-65. The next two years, technetium, molybdenum and strontium-90 will be studied. An extension will be necessary if transuranic elements are to be covered. In order to take account of seasonal differences, the studies will involve two or three types of freshwater and a range of natural and man-made products discharged into rivers and which may product synergistic phenomena with the radionuclides involved.

The sorption and desorption processes as well as the contact time will also be taken into account in order to predict the deposit distribution as a function of distance on the continental shelf.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-318-81-F

General subject of the contract:

Physicochemical changes of transuranic elements in the environment transfer through the food chain towards man.

Description of research work:

The research programme treats five distinct aspects of the environmental behaviour of transuranic elements particularly related to their transfer through the food chain. Experiments on the soil plant-animal transfer will be conducted on open field cultures. Contamination will be done by sprinkler irrigation and by soil deposits. River water will be used for irrigation at isotope concentrations not exceeding the WMPC (Weighted Maximum Permissible Concentration) in order to take full account of the weighting effect of the specific activity. The experiments will be conducted on a three year crop rotation system with three plant species: a plant with edible roots or tubers, a plant with edible fruits and a plant with edible leaves. Part of the harvest will be used as animal fodder whereas the crop residues (roots, stalks, collets, straw, etc...) will be used to estimate the isotope turnover in the soil and to assess their importance in the soil-plant transfer. The model used to study the soil parameters, leaching solutions, the effects of synergism and physico-chemical changes will be the one developed in the previous programme, namely: culture in a climatic chamber specially equipped for work on transuranic elements and samples processing in a specialized laboratory. Under these conditions the same plant species as those grown in the open field will be tested.

In the first project, synergistic effects between transuranic elements, uranium matrix and soil parameters and effects on soil-plant transfer will be tested. Previous experiments showed that significant fluctuations occured according to the relative isotopic abundance. It was suggested that this resulted from synergism related to the specific activity of each isotope. The quantity deposited also seemed to have an effect on the transfer rate. However, it is not yet possible to evaluate the interdependence between soil parameters, mobility and transfer of transuranic elements. The following aspects will thus be examined in this study: synergy between Pu, Am, Cm and Np; - synergy between transuranic elements and uranium and influence of soil parameters.

In the second project, the influence of the deposited quantity of transuranic elements on the soil-plant transfer will be evaluated. Literature data suggest that the concentration of transuranic elements in the soil determines the transfer coefficient. The purpose is to verify and to explain the relationship between deposits and transfer

and to examine the main causes of variation in soil-plant transfer. The following aspects will be developed: variation in soil-plant transfer factors as a function of deposition (1 to 10^{-10} µCi per gram of soil); variation of the localization of transuranic elements in plants as a function of deposition (root - leaf - stalk - fruit); estimate of the availability of transuranic elements in soils for different deposits by the method of successive extraction.

In the third project, a field study of soil-plant-animal transfer will be conducted, paying attention to pratical aspects of farming methods and fertilization. The test will be on processed effluents diluted to the WMPC level with river water. Two aspects will be dealt with: irrigation by sprinkling and soil deposition. The following parameters will be included in these test: mineral fertilization, organic fertilization and fertilization with plant residues. Plant-animal transfer will be examined in a specialized laboratory.

In the fourth project, the influence of uranium - sodium - transuranic matrix and glass lechate matrix on the soil-plant transfer will be studied. In order to assess the interference of the various matrices on isotope mobility in the soil and on soil plant transfer, experiments will be carried out on leafy plants (green salad) and root plants (radishes).

In the fifth project, the effect of macro- and micro-components of reprocessed waste effluents on the physico-chemical evolution on the mobility in the soil and on soil-plant transfer of transuranic elements will be tested. The physico-chemistry of transuranic elements can be modified by various chelating agents present in the effluent (carbonates, detergents, degradation products of tributyl phosphate, organic compounds found in river water and soil solutions). The following aspects will be covered: change in physico-chemical form in the presence of carbonates, detergents, organic compounds of river water and soil solution; mobility tests using the horizontal migration technique and variation in the rate of soil plant transfer of green salad as a function of the addition of various quantities of carbonate, detergent and organic compounds in waste water.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-321-81-F

General subject of the contract:

Evaluation of the impact on the population of the dispersion of Ra-226 in mining areas. In situ research on radionuclide transfer in rivers, irrigated land and processed foodstuffs.

Description of research work:

The properties of radium, the uncertainty of its behaviour in river water and the lack of information on its transfer in agricultural products make a realistic evaluation of the doses received by the population impossible. Evaluations thus tend to be based on conservative hypotheses. Knowledge of the rates of transfer in agricultural products from the South of France is not without interest to the Community since these regions export grapes, wine fruits and market garden produce to other Community countries. The programme covers several stages:

(1) Examination of cultures,products consumed and processing of foodstuffs, production and consumer markets affected in Europe.

(2) Determination of the principal transfer coefficients in freshwater, irrigated cultures and fresh or processed agricultural products. Field observations of radium distribution between water, suspended particulates sediments, fauna and flora at certain distances from the point of discharge and at different times will be used to define more realistic distribution coefficients than those obtained from experimental models since they incorporate all the dynamic seasonal and physico-chemical parameters, which cannot be implemented experimentally. For irrigated cultures, the transfer coefficients will be determined at mining sites with emphasis on crops for which detailed field studies have not been carried out up so far, such as vines, or on crops for which special farming techniques, such as irrigation by sprinkling or flooding are applied. An estimate of agricultural production and sales on European markets are the parameters which will determine the cultures and the particular points of sampling. The effects of food technology will also be studied from field samples both before and after the application of the main types of food processing (physical treatment, the various types of cooking and fermentation). The main products marketed and exported will also be examined, e.g. wines.

(3) Existing transfer models will be revised in the light of the observations made. They will then be used, including the transfer coefficients determined in this study, for the computation of population doses.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-315-81-F

General subject of the contract:

Transfer and evolution of certain long-lived beta emitters in terrestrial food chains leading to man.

Description of research work:

The research programme is subdivided into three projects respectively dealing with technetium-99, iodine-129 and selenium-79, nickel-59. This programme is an extension of research work on the transfer of isotopes in the terrestrial environment that has been pursued in this laboratory for some 20 years. This work has led to the development of techniques and simulation methods required for such work. With regard to long-lived beta emitters, certain problems have not yet been solved, in particular their specific activities. These problems involve : chemical and microbial toxicity in the soil and plants; long-term field-scale transfer from deposits obtained by irrigation with water at Weighted Maximum Permissible Concentration (WMPC); synergism in soil/plant transfer (gas entrainment, effect of temperature, type of contamination, irrigation and application of sludge); and food chain transfer to animals from field crops. The three projects address problems involving isotopes which enter the environment through the atmosphere or in liquid form and which are also present in storage facilities. Experiments on the soil-plant-animal transfer will be conducted on open field cultures. Contamination will be done by sprinkler irrigation and by soil deposits. River water will be used for irrigation at isotope concentrations not exceeding the WMPC in order to take full account of the weighting effect of the specific activity. The experiments will be conducted on a three-year crop rotation system with three plant species : a plant with edible roots or tubers, a plant with edible fruits and a plant with edible leaves. Part of the harvest will be used as animal fodder whereas the crop residues (roots, stalks, collets, straw, etc) will be used to estimate the isotope turnover in the soil and to assess their importance in the soil-plant transfer. In the first project, technetium will be studied in a soil-plant-animal system. Various aspects will be examined :
chemical and microbial toxicity, volatilization from soil deposits and during decomposition of crop residues, deposits in the presence of HF in order to simulate discharge from UF_6 fabrication and enrichment plants, technetium/molybdenum synergistic effects on root absorption, transfer of technetium from animal fertilizers and transfer through tobacco and cigarettes. In the second project, special studies will deal with iodine. The following phenomena will be examined : volatilization from leaf deposits, absorption by certain aquatic plants

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(such as cress), absorption by oleaginous plants (production of oils), effects of fertilization on volatilization and transfer through tobacco and cigarettes. In the third project, some aspects of the behaviour of selenium-79 and nickel-59 will be studied : transfer after application of residual sludge generally containing high levels of Se and Ni, transfer of selenium in protein-bearing-plants, effect of sulphate containing fertilizers on the transfer of selenium and
effect of potassium and phosphate fertilizers on the transfer of nickel.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-316-81-F

General subject of the contract:

Actinides in the marine environment : study of their physico-chemical behaviour in seawater and marine sediments and their transfer between sediments and benthic species.

Description of research work:

The aim of the research programme is (1) to study the physico-chemical behaviour of actinides, primarily plutonium, americium, and neptunium, in seawater and in marine sediment, and (2) to examine the potential for transfer of actinides from contaminated sediments to benthic organisms. The emphasis work will be on slowly evolving long-term phenomena, which are crucial in this study. The research work will be carried out essentially in the laboratory, and the results will be compared with data collected on field samples.

1. Physico-chemistry of actinides in seawater and transfer between water and sediment. The physico-chemical studies will be concerned with the possibility of containment of actinides in marine sediment. The problem is to establish the actinide concentration capacity of different types of sediment and to determine whether actinides are fixed indefinitely in the sediment or whether they can released by chemical modifications in the environment. The two following approaches will be adopted : (a) study of the sorption of actinides on marine sediments and of the sorption kinetics of various types of sediment, study of the sorption of specific chemical forms such as hydrolyzed or chelated forms, influence of the chemical composition of sediment, in particular its iron and organic matter content : (b) study of the desorption of actinides from the sediments and measurements of distribution coefficients between sediment particles and interstitial water, impact of progressive chemical modifications of the interstitial water, influence of variations of the oxydo-reduction potential, effects of sulphides and by-products of bacterial metabolism, effect of petroleum hydrocarbons.

2. Transfer of actinides to benthic organisms. The studies will deal mainly with the direct transfer of actinides from contaminated sediment to benthic flora and fauna. The species taken into account will be : (a) suspensivorous species which filter the suspended sediment particulates in the sea water. An example would be Mytilus edulis mussels which are widely found and consumed by man. This species derives its interest from the fact that it has been selected in a large-scale international programme of marine pollution monitoring (Operation Mussel Watch).

(b) limivorous species which live in the deposited sediment and ingest silt particles. These could be bivalves such as cockles

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(Cerastoderma edular) and certain types of polychaeta (Nereis, Capitella capitala) The polychaetes are interesting by the abundance of their biomass and because they constitue one of the first trophic levels in marine food chains.
These species are raised in aquaria on artificially contaminated sediments under controlled conditions to ensure an equilibrium of the environment and an adequate survival rate. The actinide concentrations will be measured periodically in the living species, in the sediment and in the interstitial water. A comparision of these concentrations will enable to identify the transfer pathways and to evaluate the corresponding concentration factors. The results obtained will be compared with results obtained elsewhere on field samples.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-B-438-81-UK

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General subject of the contract:

Cellular biochemistry of uranium, plutonium, americium and curium in the common marine mussel Mytilus edulis L.

Description of research work:

Research already in progress at IMER, Plymouth, aims to describe and account for the distribution of uranium and some (Pu, Am and Cm) transuranium radionuclides in the marine mussel, Mytilus edulis. These elements are available for entry into the animal by uptake from either seawater or food in a variety of chemical and physical forms. In this project measurements are made of the various radionuclides in total tissues, parts of organs, and sub-cellular fractions in order to identify major routes of entry, sites of deposition and retention rates for whole animals and various organs; these data are used to describe distributions in relation to active or passive associations with defined biochemical processes. In addition other related studies are also being investigated; these concern the concentrations and form of uranium, plutonium and americium in seawater, marine suspended particulates, items of diet and resuspended surface sediments. The present proposal is to add three essential elements to this work at levels of more rigorous examination, namely :

(i) To establish at the cellular level whether or not uptake by tanned proteins of the mussel (e.g. byssal threads, periostracum) is passive involving cell surfaces, or active following transfer of the radionuclides into the systemic circulation of the animal.

(ii) To study the importance of chemical form for conservative and non-conservative species in relation to uptake across the gills, the digestive tract, and mode of excretion of incorporated forms through the excretory system of the animal (i.e. kidneys and pericardial gland). Emphasis will be placed on distributions in cells.

(iii) To study the role, if any, of the animals lysosomal-macrophage system in removal, or sequestering, relevant radionuclides in cells of the digestive gland, kidney pericardial gland. As far as possible all three elements of this programme will be studied in natural environments, but supported when essential by laboratory aquaria experiments. Mussels will be studied in two types of environment, namely:

(a) From the Esk estuary, Cumbria, UK. where for several decades they have been exposed to effluents containing U, Pu, Am and Cm from the British Nuclear Fuel Ltd nuclear fuel reprocessing plant, Cumbria, UK.

(b) Sites in SW England which receive transuranics from atmospheric fallout and uranium from normal, and elevated terrestrial runoff as a result of local natural ore mineralisation.

Each environment contains source materials in different chemical forms

and evidence exists to indicate that for each uptake and distribution within the mussel is different. The concentration of transuranium radionuclides in environment (a) is about 30 pCi g dry wt in (b) about 0.3 pCi g dry wt. In neither environment can the levels of exposure give rise to chemical toxicity; effects from ionizing radiation are not likely to be seen but they will be considered, especially in tissues adjacent to hot particles found in the intestine and pericardial gland. In a 12-month period measurements will be made to cover two conditions:
(i) Animals in a ripe reproductive state when total body weight is at a maximum and transfer of radionuclides to gonads may be examined.
(ii) Animals post spawn (early summer) when it will be possible to examine detrital levels when macrophage activity is elevated. All studies will consider the intracellular concentration and distribution of the various radionuclides with special attention being given to any association with defined biochemical processes at organ or cellular levels. In each study animals will be dissected and prepared for examination soon after collection. The following tissues will be dissected and analysed for concentrations and distributions : gills, digestive gland, kidneys, pericardial gland and mantle. Methods of analysis will be based upon conventional chemical procedures and surface barrier alpha spectrometry; a recently developed new di-electric detector, CR-39 in conjunction with micro-thin layer chromatographic procedures, will be used for samples containing very small quantities of Pu, Am and Cm; the delayed neutron and fission tracks technique will be used for uranium analyses when appropriate.
(iii) Preparations of lysosomes (and other organelles e.g. nuclei, mitochondria, endoplasmic reticulum and residual materials such as lipofuschin) from the digestive gland, kidneys and pericardial gland using techniques available at IMER, followed by radionuclide assay using an appropriate method. Parallel studies will be made to identify the distribution of some organic associations (e.g. -SH bonds; electron dense granules in the digestive gland, kidney and pericardial gland; amino-acids) in relation to the presence of radionuclides by conventional microchemical and histological techniques. During these investigations other studies, not the subject of this proposal will be carried out to identify the extent to which the behaviour of the U, Pu, Am and Cm may be described in terms expected for other elements having the same general chemical characteristics (e.g. Rare Earths), or whether they possess special properties and hence have no true stable element analogues; this work will be restricted to the Esk estuary and will consider distributions of Zr-95, Ce-144 and other radionuclides derived from BNFL effluent. This proposal is divided into two projects, the first is concerned with the cellular distribution of actinides in the mussel, the second deals with more general matters with an objective of providing a model to account for transfer of the actinides from the natural environment, their retention times in the mussel and behaviour in nearshore and estuarine ecosystems. Attention will be focussed upon identifying the relevant biochemical pathways involving the actinides in terms of major processes.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-B-327-81-B

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General subject of the contract:

Measurement and analysis of the evolution of the krypton-85 activity in the atmosphere and study of its synergistic action with chemical pollution.

Description of research work:

It is intend to follow-up the krypton-85 concentration in the atmosphere by frequent air sampling (at the laboratory site) and measurement of the activity. Therefore the existing set-up for air sampling, cryogenic distillation, chromatographic separation with use of a hot-wire detector, mass determination with use of a precision pressure gauge, and counting with a liquid scintillation counter will be duplicated, and a mobile compressor for sampling in other places will be installed. In cooperation with other laboratories specialised in the field of meso-scale atmospheric transport, it will be attempted to relate the measured Kr-85 concentrations to the source terms. On the one hand the magnitude and time profile of excess activities measured after a known release to specific meteorological conditions will be correlated and on the other hand the agreement of probabilistic meso-scale transport models with the total number of measurements will be examined. As a first step a backtracing will be performed from the dates on which an excess activity is measured, in order to determine the main source site(s).
The collective doses to the European and world populations from measured and computed Kr-85 activities will be derived. Therefore an accurate calculation of the dose from external beta-radiation will be carried out and attention will be paid to the eventually synergistic biological effects of radiation and U.V. exposure of the skin.
In view of the eventual synergistic effect of radiation and conventional pollution, inducing acidification of the environment and smog, the frequency of high Kr-85 concentrations will be estimated thoroughly.
In the first phase of this project the processes of homogeneous nucleation and of nucleation around ions will be studied in a thermostatic flow reactor, in which pure air with controlled concentration of SO_2, NO_2 and H_2O are introduced. The reactor can be U.V. irradiated and can be exposed to ionizing radiation by an external gamma source or by a Kr-85 source in line. Downstream the reactor the particle concentration will be measured with a continuous condensation nucleous counter and the particle charge with an ion trap. A chemical analysis of the aerosol with PIXE (particle induced X-ray emission) will be performed. The dose rate dependency of the effects will be

studied under static conditions in a 20 l vessel with use also of other radiation qualities to allow a microdosimetric interpretation of the effect.
In a second phase of the project the particle size distribution as a function of the discussed parameters will be measured; the moments of this distribution determine the properties of the aerosols and their precursors.
Distribution measurements (range < 1 μm) will be done in a 100 l vessel with use of a diffusion battery and of an electrostatic classifier coupled to the condensation nucleous counter and the evolution of the gas concentrations (SO_2,NO,NO_2,O_3) will be followed.
Finally the effect of Kr-85 in a real environment will be studied by using two transparent Teflon balloons, exposed to sunlight, and filled with unfiltered air, eventually enriched with SO_2. In one of the balloons realistic amounts of Kr-85 will be added. In this phase of the project the distribution measurements will be extended above 1 μm.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-313-81-D

General subject of the contract:

Distribution of tritium- and carbon-14-compounds over the aqueous and organic phases of various structures of aquatic and terrestrial food chains.

Description of research work:

The extent and type of link in the various organisms of the food chains play a major role in the exposure of humans to radiation through the radionuclides tritium and carbon-14. This calls for a study of the biological behaviour of the various tritium or carbon-14 compounds in the different types of fresh water and sea water fauna and also for an inventory of the radionuclides deposited through primary production in a form that can be transported via the food chain.

The food chain in question would be phytoplankton (green algae) zooplankton (Daphnia), fish and man.

Through the photosynthesis of the plant cell, water including tritiated water when present, forms organic compounds with atmospheric carbon dioxide or water dissolved carbon dioxide, both eventually with carbon-14 dioxide. Model experiments designed to establish the kinetics of the incorporation of tritium have shown that there is discrimination between tritium and protium. To what extent this also applies to the various carbon isotopes (C-12,C-13, C-14) is currently being examined. It is not known with certainty what metabolic steps are involved in this process, but it is known that certain enzyme reactions of the intermediate metabolism are sensitive to isotope differences of hydrogen in particular. Increased tritium activity were found in citrate, aspartate, glutamate and glutamine. It is also known that intermediate products of the cycle of Krebs, such as aspartic acid via inosinic acid and orotic acid combine with the pyrimidine nucleotides of nucleic acids. The purine nucleotides are formed around the glycine molecule with the help of "active methyl groups" and fractions of amino acids of the intermediate metabolism. The extent to which organically bound hydrogen is passed on in this process together with the carbon skeleton, leading to enrichment, is still to be studied.

Structures with a lower conversion rate of the fixed hydrogen than water are formed from proteins. The incorporation of tracer compounds and their transmission are possible in the form of essential amino acids. The determination of transfer data from one step to another is the first stage towards providing a general picture of the incorporation of tritium and carbon 14 in organic substances.

The following phases will be examined in the nuclide uptake by phytoplankton : a) incorporation of tritium and carbon-14 through the photosynthesis of various algae (eucaryotic fresh water or sea-water algae, blue-green algae) in model systems; b) ratio of radionuclide

given in organic and inorganic form; c) establishment of the components capable of being passed on as food steps (precursor) under physiological conditions; d) assessment and quantitative determination of the metabolites formed and their degree of contamination.
With regard to the uptake of nuclides by aquatic zooplankton (Daphnia), green algae are their main food. The following individual phases will therefore be examined at this level: a) feeding of daphnia with labelled substances and determination of the incorporation of tritium or carbon-14 into the body substance of lower crustaceans; b) determination of the tritium and carbon-14 precursor found both in the intestinal tract and body substances of daphnia in view of their utilization in the following step of the ecological chain.
With regard to the uptake of nuclides by fish consumed by humans, phyto- and zooplankton are the initial and intermediate steps in the food chain. While carbohydrates and fats are largely burnt up to form CO_2 and water in the production of energy, the protein components reach the resynthesis mechanism of fish protein through amino acids. It must be established to what extent tritium carbon-14 labelled structures are retained in this process. The following tests are to be carried out : a) feeding tests on fish with labelled daphnia; b) assessment of the preliminary metabolic products labelled in fish which can be consumed by humans.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
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Contract no.: BIO-B-431-81-B

General subject of the contract:
Simulation of tritium transfer in the environment : study of organic tritium transfer in the aquatic environment, the soil-plant and the plant-mammal system.

Description of research work:
The proposed study contains four research projects intended to provide maximum comprehension of the tritium cycle in the environment. Given the complexity of the tritium cycle, the most rational approach likely to lead to an overall understanding rather than partial incomplete knowledge is to combine both modelling and experimentation. This approach assumes that the model is initially at a slightly more advanced stage than the experimental work and that a continual feedback between research workers engaged in designing models and those engaged in field and laboratory experiment is set up. The four interrelated projects involve the modelling of the behaviour of tritium and an experimental study of the transfer of organic tritium in the aquatic environment, the soil/plant system and the plant/mammal system. These projects might possibly be completed by contributions from teams working on the conversion into organic form of tritium in the soil and on tritium exchanges between the soil and the atmosphere.
1. Modelling of the behaviour of tritium. Description of this research programme is given under contract number BIO-B-340-81-F.
2. Behaviour of tritium in different chemical forms in the freshwater environment. Information on the uptake of tritium in single or multicellular aquatic organisms is not yet sufficient to provide an understanding of the tritium cycle in this part of the environment. To obtain a real assessment of the tritium turnover in aquatic organisms it is required to have data not only on the quantity of radioactivity absorbed and metabolized by the cells, but also the activity discharged into the environment, essentially in organic form. Information is currently available on the excretion of tritiated biological molecules by living organisms, and, to bridge this gap, a study is needed regarding the incorporation and discharge of tritiated organic substances by fresh water algae such as Chlamydomonas cultivated under dynamic exchange conditions. The experimental specifications and analysis procedures will be specified in conjuction with the team responsible for the simulation model.
3. Behaviour of tritium in the soil-plant system. Data have been collected so far on the fixation of tritium into plant water by evapotranspiration and into the organic matter of plants by photosynthesis, but only part of these data can be used in tritium transfer models. In addition, there is a lack of data on the transfer of organic tritium in the soil-plant system. The organic matter of soil

and plant are composed of different types of molecules with different turnover characteristics. The transfer rates between soil organic matter and plant organic matter are not known; and it is not certain whether several categories of organic matter should be considered both in plants and in soil. It is also unknown whether several soil layers should be distinguished. The answers to each of these questions must should lead to a specific experimental approach.

4. Behaviour of tritium in mammals. Description of this research programme is given under contract number BIO-B-432-81-NL

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-467-81-B

General subject of the contract:

Technetium transfer in animal food chain

Description of research work:

The experiments proposed aim to evaluate the transfer of technetium in the animal food chain, the influence of thyroid activity and dietary iodine, and the chemical toxicity of technetium.

a) Determinatium of transfer coefficients of technetium

Sheep will be exposed to food contaminated with Tc-95m-pertechnetate labeled with Tc-95m (half life 61 days). The excretion products, feces, urine and milk will be assayed for radioactivity. Animals will be sacrificed 1,3,7,30 and 100 days after application, and retention and distribution among organs will be determined. Uptake by the thyroid will be monitored by external counting, and the thyroid will be studied for histopathological lesions at sacrifice. As the uptake of technetium may depend on the composition of the diet (intestinal microflora), the total amount of the technetium will be varied, and experiments will be carried out with sheep kept on dry or fresh feed. Cows will later be utilized to obtain specific information for other types of lifestock.

These experiments will eventually be complemented by studies on sheep (initially on rabbits) in which Tc-99 is given as plants contaminated with technetium during growth. Such material will be obtained by cooperation with CEA Cadarache.

b) The influence of thyroid activity and dietary iodine.

The influence of different amounts of iodine in the diet on the uptake distribution of technetium will also be followed in sheep.

c) Chemical and radiological toxicity of technetium.

Metabolic data, such as obtained by the experiments described above, allow to assess the amount of technetium transferred via food pathways and eventually reaching man. An evaluation of the hazards of technetium requires, however, in addition the determiantion of the chemical and radiological toxicity of technetium. It would, thus, be desirable to study the effects of a continuous dietary supply of technetium in the rat with particular emphasis on the thyroid (uptake of radioactive iodine, blood levels of thyroid hormones, histopathology of the thyroid). The effect of normal or iodine deficient diets containing different amounts of Tc-99 might be compared with that of a diet containing Tc-97 with a ten times longer half life (2.6×10^6 years) and a correspondingly lower radiological toxicity but the same chemical toxicity. In this way, one could evaluate the chemical toxicity of

technetium. In addition, one could compare the effects of technetium irradiation with that delivered externally by X-rays or internally by radioactive iodine.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-314-81-D

General subject of the contract:

The influence of topography on the dispersion of nuclear pollutants, exchange processes of HTO vapor between soil and atmosphere and accumulation of I-129 in the human thyroid.

Description of research work:

1. Investigations of the influence of topography and surface roughness on the dispersion of nuclear pollutants.

The increasing burden of the atmosphere with nuclear pollutants demands a detailed investigation of the influence of topography and vegetation on the dispersion behaviour of nuclear aerosols. In specific it is important to study how the surface roughness and the boundary layers of vegetation and buildings effect the aerosol spectrum, as the size distribution of the particles is a major parameter in their propagation mechanisms. Little is known about the influence of topography on the behaviour of a cloud of harmful substances, therefore, the fall-out and deposition of aerosols in the lower atmosphere cannot be described satisfactorily. In order to investigate the dispersion of aerosols under all natural and artificial influences, a pyrotechnical aerosol generator was developed which produces aerosols labeled with easily activables tracers. (This is necessary, as the application of radioactive substance in the biospere is only possible with few shortlived radionuclides unsuitable for long-term experiments over longer distances). The simple generation provides a high mobility of the source for an application in the atmoshpere. The labeled particles can be detected with high sensitivity by activation analysis. The labeling guarantees a clear identification of the origin of the particles, which are collected by cascade impactors. The measured size distributions are evaluated with respect to the given topography. The aim of this research is to determine the influence of surface structure on the dispersion and on the size distribution of aerosols in the range between about 0,5 - 7 µm.

2. Exchange processes of HTO-Vapor in the soil-plant atmosphere system.

In the past years special measuring methods were developed to trace the exchange of H_2O an HTO-Vapor between atmosphere and soil and the movement within the soil. The nuclear and non-nuclear techniques have been tested in laboratory and field experiments, and preliminary results have been obtained. Investigations are to be concentrated on possible pathways of atmospheric HTO from natural and artificial sources, entering the soil layers, which are involved in the condensation and evaporation processes during a day-night cycle, and on the meteorological and soil-physical conditions governing this

transfer. Experiments will be carried out in the laboratory under simulated conditions to determine the effectiveness of single parameters. Field experiments are taken into consideration. Further studies will be devoted to the influence of the temperature and moisture regime of different soils on the transfer of HTO-Vapor and the exchange of HTO and H_2O within the soil, yielding information on fractionation and isotope effects. Finally, the uptake and incorporation of HTO in the vapor and liquid phase into selected plants will be examined. The combination of the transfer between all the compartments involved will provide the basis for an estimation of the tritium transfer into the biocycle.

3. Investigations on the accumulation of Iodine-129 in vegetation, milk and human thyroid glands in the vicinity of nuclear facilities.
From the investigations on the I-129 content of animal thyroid glands from the vicinity of nuclear facilities the direct danger for humans cannot be deduced. The results do not represent the accumulation of I-129 in human organisms. A direct determination of I-129 in human thyroid glands has not been carried out yet, at least not within the area of the European Communities. Information on the I-129 concentration in vegetation and milk, to at least roughly estimate dangers to man, are also missing. Therefore, the suggested project includes a systematic study of vegetation milk and human thyroid glands. There should be a great number of samples taken from an area within a limited time intervall an investigated for I-129 contents. Only in this manner statistically reliable mean values of all three compartments can be obtained yielding information on the transfer of I-129 and its accumulation in man. The transfer factors provide a basis for the determination of the risk to human organism from the I-129 content of milk at any later point of time.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-328-81-B

General subject of the contract:

Hydrological and biogeochemical characteristics which condition the environmental behaviour of the mobile long-lived radionuclides. Application to the ultimate biological availability of Tc.

Description of research work:

A good deal of european nuclear installations will be located in natural regions similar to what may be found between Normandy and the Eifel i.e. in forested humid temperate regions sparsely populated where most of the arable land is under permanent pasture, the soils having generally a high colloid content. Any local study has to be carried out in sufficient depth so that its results may be generalized without undue difficulty with a minimum number of local determinations. The possibility of carrying such a study exists in a site typical of the conditions in NE france or SE Belgium in the Fagne de Chimay where this University has established a field laboratory in 1975. It is suggested to use this laboratory and the facilities and expertise available at the main laboratory for carrying out a study of a small watershed from the point of view of the mobility of elements in a well defined ecosystem.

In the first phase, one would gather the usual data base for such a study regarding climate and hydrology. In the second phase, one would define compartments amenable to study for the various constituents and try to determine their size and transfer coefficients between them. A study will be made of fluxes of various elements chosen not so much from their ecological importance but from the point of view of the information which could be obtained for the mobility of radionuclides in the environment using the analog element approach. These fluxes would be studied between the forest cover the permanent pastures, soils and surface water.

The data will be integrated in a quantitative model the components of which would be submodels describing some of the important process in a as realistic manner as possible. These models should be enlarged to include elements without ecological significance but possible radiological hazard.

Using the information gathered as mentioned above the prediction of the way in which radionuclides would be dispersed in the environment would be based on the analog-element approach concept, which will be applied to determine the ultimate biological availability of Technetium.

There is very little data regarding the long-term biogeochemical behaviour of long-lived radionuclides in the environment and specially regarding the environmental behaviour of mobile long-lived

radionuclides like Tc-99. Their process of transfer cannot only be understood from a simple extrapolation of the results obtained from short-term experiments conducted in the Laboratory and some of the data which are available need to be improved in reliability. One new and constructive approach consist in utilizing naturally occurring elements which have chemical properties very much like the retained radionuclides (analog element-approach) and which have reached an equilibrium. Recent experiments have confirmed that the long-term behaviour of these elements may be modeled effectively by the behaviours of well-selected natural occuring analog elements.
Tc may largely bioaccumulate in plants from soils (transfer factors reaching more than 100) and plant exhibits localization in leaf tissues immediately after exposure. The toxicity symptoms, linked to biochemical rather than radiation toxicity, would be due to Tc substitution for a nutrient element ($H_2PO_4^-$; SO_4^{2-} ; MoO_4^{2-}) or to an inactivation of an essential plant process.
In aerobic soils Tc may be predicted to be highly mobile (short-term behaviour) but physico-chemical, biological and time factors may be important in reducing its mobility. The soil organic fraction may be expected to modify its mobility and may lead to its immobilization through sorption, reduction or complexation. Moreover, over longer periods, the soil microflora may account for additional sorption and reduction (Tc VII - Tc IV ; direct or indirect). Tc retention also appears to be closely related to the amorphous Fe and Al fraction of the soils. In flooded soils (permanent and non permanent soils), the reducing environment may result rapidly in formation of highly insoluble $TcO_2.2H_2O$ or sulfides (Tc_2S_7 - TcS_2) or coprecipitation of Tc (IV) with other insolubles such as Fe sulfides. These soils have potential for serving as repositories for Tc and rotations may make it again available for plants. Molybdenum and Rhenium will be considered as potential Tc analog and their environmental behaviour will be studied.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-436-81-F

General subject of the contract:

Study of the physicochemical properties of alpha and beta emitting aerosols aiming at the measurement of the associated nuisances.

Description of research work:

Aerosols found in the working atmosphere may have the following origins: 1. Production and emission of solid or liquid aerosols during various mechanical and chemical operations on flammable materials and waste. Once suspended in air the largest fraction deposits rapidly, contaminating installations and equipment, whereas the finest fraction can be inhaled by the workers. The degree of nuisance in the latter case is probably linked to the amount inhaled. 2. Production of ultrafine radioactive particles from the disintegration of natural (radon, thoron, etc.) and artificial (Xe, Kr, etc.) radioactive gases. The dynamic behaviour granulometric changes and elimination) of these particles depends largely on the concentration and dimensional distribution of the aerosol. The nuisance in this case is linked to the fine fraction diameter less than 0.2 μm of the aerosol which can be inhaled and deposited by diffusion. Precise knowledge of the nuisance presented by radioactive aerosols, i.e. deposition (risk of external beta and gamma irradiation) and inhalation (risk of internal alpha irradiation) requires detailed knowledge of aerosol granulometry, dynamics and state of electric charge in the work place. This knowledge is also indispensable for evaluating errors made in the selection of aerosol sampling and measuring equipment used for radiological surveillance. Priority is given to the following work places:

a) underground and open cast uranium ore extraction. Two radioactive components exist, one due to the radon daughters whether fixed or not on atmosphere particulates and one due to ore dust;

b) drying and barrelling of uranate in uranium ore processing plants. The nuisance presented by the granulometry of the suspended uranate powder can vary considerably with the type of fabrication, drying and barrelling;

c) certain workshops or laboratories in any irradiated fuel reprocessing plant where various highly specific aerosols, such as plutonium, can be found. At the same time studies on a simulator will be undertaken to determine certain aspects of dimensional form or state of electric charge.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. J. Maisin
Département de Radio-
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Boeretang, 200
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Contract no.: BIO-B-330-81-B

General subject of the contract:

Study of the impact of waste from PWR nuclear power stations on the fresh water ecosystem.

Description of research work:

The results obtained up so far of the effects of the Tihange 1 nuclear power plant (PWR, 870 MWe) on the River Meuse give a better indication of what direction research should take now. It was evident on several occasions during the first phase of the programme that the research undertaken by the various teams involved was not sufficiently oriented towards a study of the impact of waste on the fresh water ecosystem. This involves a study of the trophic structure at all levels: from primary producers to fish, including decomposers. In addition, there is strong indication that the effects of a slight increase in water temperature are reflected more at the functional level (activities of decompositon, photosynthesis, respiration) than at the level of the biomasses or structure of the aquatic biocenosis. The transfer mechanism of the radionuclides to the various compartments of the river ecosystem (partly transferred directly from the water and partly transferred within the trophic chain) should be studied along with the kinetics of accumulation. In this contest the research programme is as follows :

1. Continuation of the current phytoplankton programme in order to obtain results over several annual cycles; biomass measurements, quantitative and qualitative analysis, measurement of synthetic activity, measurement of radionuclide concentrations. Biomass measurements of periphyton should also be continued upstream and downstream of the plant in question.
2. In situ studies, including physicochemical analyses of the water and an analysis of the microscopic algae and bryophyta populations, will be continued. The frequency of sampling and analysis of the parameters linked to the temperature increase of the water and to the organic pollution (temperature, dissolved oxygen, pH, phosphates, nitrates, nitrites, ammonium compounds and total carbon) will be bimonthly.
3. The study of the radionuclide accumulation in primary producers forming part of the food of plant-eating animals will be intensified. In particular, the mass culture of bryophyta immediately downstream of the plant discharge point will be developed to gain a better picture of the accumulation kinetics of radionuclides found in the liquid effluents of PWR power stations.
4. The study of the toxic effects of the biocides used in the power station on organisms representing the various trophic levels will be

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Continuation contract no.: BIO-B-330-81-B

intensified and any synergistic effects will be studied (algae, aquatic species).

5. The study of the growth and reproduction of fish (roach being the most representative species) will be continued since these functions depend directly on the water temperature. Moreover, the commissioning of phases 2 (900 MWe) and 3 (980 MWe) of the Tihange plant will have an effect on the temperature regime of the Meuse.

6. A new research project in order to identify the sources of organic tritium observed in some effluents from the primary circuit is planned. The principle of this work consists in the reproduction in laboratory conditions of the different steps that might generate tritiated organic compounds, with high specific activity, followed by the determination of the biological availability with the help of the Scenedesmus culture which is now used on a routine basis for the detection of tritiated organic substances in solution.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. I. R. McAulay
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Contract no.: BIO-B-338-81-EIR

General subject of the contract:

Measurement of levels of radioactivity in the marine life and waters of the Irish Sea and their contribution to radiation dosage of the population.

Description of research work:

Radioactive materials are discharged into the Irish Sea as a result of the operation of the Windscale nuclear reprocessing plant and also from a number of other installations using nuclear materials. These discharges give rise to measureable levels of some radioactive isotopes both in the waters of the Irish Sea and in the marine life which exists there.

A number of these isotopes, notably caesium-137, caesium-134, and zirconium-95 are present at concentrations which may under some circumstances be of importance in their contribution to the radiation dosage to the population. Measurements will be made of the concentrations of the gamma emitting radioisotopes present both in the water and in a number of specimens of the marine life of various species. These specimens will include edible fish, benthic dwellers and a number of seaweed varieties. The sampling will be done with the advice and assistance of the Irish Fisheries Research Laboratory, and it is expected that the data obtained will enable concentration factors to be determined for a number of radioisotopes. Information will also be gained concerning pathways leading to the uptake of radioisotopes by man.

The equipment used will be a scintillation crystal viewed by a photomultiplier and feeding its output to a multichannel analyser. A second crystal, of the well type, will be used for the examination of the activities of samples obtained from sea water using ion exchange resins. Both scintillation counters will be operated with substantial lead screens.

The preparation of samples will be in accordance with procedures used in other laboratories and arrangements will be made to use intercomparison samples in conjunction with other laboratories. It is hoped to make measurements of the total beta activity of samples at a later stage in the project, but it is not at present proposed to carry out measurements on transuranic elements which may be present in the Irish Sea. From the measurements to be carried out in this project, it will be possible to assess the contribution to radiation dosage to the population as a result of waste disposal into the Irish Sea. As a secondary aim, it is hoped to gain some information on clearance times and circulation mechanisms for the Irish Sea.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-331-81-UK

General subject of the contract:

Behaviour of radionuclides in the marine environment in support of the disposal of wastes arising from the utilization of nuclear energy.

Description of research work:

Two aspects of waste disposal to the marine environment which require a more detailed study are:
1) the behaviour of long-lived radionuclides in coastal waters.
2) the behaviour of radionuclides in the deep sea.
The former is required because it is in such areas that most discharges are made; the latter to improve our assessment of the disposal of low-level packaged wastes on the ocean bed, and to assess the potential of the deep sea to receive high-level radioactive waste.
In support of these requirements, two separate projects are carried out :
1) The behaviour of transuranium nuclides, and selected long-lived fission product nuclides (such as Tc-99), which are present in the Irish Sea as a result of the authorized low-level liquid discharges from the BNFL fuel reprocessing plant at Windscale. The emphasis in this project will be placed on understanding the mechanism responsible for the observed behaviour so that the data obtained will be more generally applicable rather than relate to one specific coastal region.
2) A more general study of deep water radioecology by initially analysing the deep sea fauna, sediments, and water for selected naturally-occurring radionuclides. The project will be so designed that the data obtained could prove useful to predict the behaviour of other nuclides in such an environment, and also to assess the existing radiation regimes to which the deep sea fauna are already exposed.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-334-81-UK

General subject of the contract:

The remobilisation of actinides from contaminated intertidal sediments.

Description of research work:

The role of sediments as a sink or trap whereby actinides are removed from solution and become immobilised has been known for some time. However there is little information on the release of actinides from surface sediments in the intertidal zone.

Preliminary field studies in which the salinity and actinide concentration in filtered water were compared, have indicated that plutonium and americium may be leached from sediments in the Ravenglass Estuary of West Cumbria, by fresh water at low tide and by brackish water backed up by the incoming tide. The contribution of plutonium-239 in solution, apparently remobilised from sediments, varied between 0.1 to 0.4 pCi l^{-1} depending on the state of the tide.

It is proposed that further field studies be undertaken to confirm both the presence of the remobilisation effect and its magnitude. Initially these will be confined to the Ravenglass estuary but depending on the results, may be extended to other areas including the Duddon estuary and the Solway Firth. The field work will be complemented with laboratory studies the results of which should indicate possible mechanisms.

Field experiments.

(1) The collection of samples of estuarine water throughout a full tidal cycle and their analysis for salinity, plutonium-238 and-239/240 and americium-241, in order to confirm the preliminary findings.

(2) Further confirmation would be obtained by the collection and analysis of samples of fresh water at low tide from several points between the head of the estuary and its mouth.

(3) Comparison of the results from (1) with measurements on samples collected concurrently at the mouth of the estuary, should indicate whether the actinides remobilised in fresh water are subsequently removed from solution in the presence of seawater. This will enable the net effect of remobilisation within the estuary on the concentration of actinides in water leaving it, to be established.

Laboratory studies.

These will involve a series of experiments with contaminated sediments in which the estuarine environment would be simulated under controlled conditions. Leaching studies will be carried out with

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Continuation contract no.: BIO-B-334-81-UK

both fresh and seawater on sediments (a) continually covered and (b) intermittently exposed to the air. The effects of such parameters as pH, water quality, temperature and light exposure on remobilisation will be studied, together with the oxidation states of sorbed and desorbed plutonium.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-332-81-UK

General subject of the contract:

Measurement of particulate airborne radioactive materials.

Description of research work:

Because of the current awareness of the problems of radiation and radioactivity and the necessity of adding internal committed dose equivalent to external dose equivalent (ICRP 26) it is essential to improve methods of determining the committed dose equivalent (CDE). At low levels biological monitoring is not possible and reliance must be placed on the measurement of airborne concentrations and the calculation of the CDE. It is also necessary to measure accurately the efficiency of samplers in outlet stacks to determine the activity released.

This work programme is concerned with the effect of external airflow on the efficiency of air sampling devices both indoors and out-of-doors, the measurement of particle dispersion and concentration and the effect of varying ventilation patterns. This will enable better estimates of concentration of breathed aerosol to be made and the CDE to be reduced by improving the performance of alarm samplers and by minimising the concentration gradient between the breathing zone and the sampler.

The work will be directed to producing equipment and methods which are of general use in laboratories and plants handling radioactive materials. Various operating plants will therefore be visited to obtain information and to apply the results of this study to practical problems.

a) Effects of external airflow

The effect of particle size and external airflow on the efficiency of a wide variety of air sampling devices will be determined. Personal, area, alarm outdoor and stack samplers will be studied. Sampling heads will not be used in isolation but will be attached to samplers, models of people, walls etc to simulate operational conditions. Improvements in design will be made where possible. The work will be performed in a wind tunnel designed for the purpose. Monodisperse aerosols suitably labelled (by radioactivity or other means) will be used to determine the efficiency versus particle size characteristics. Deposition losses in the sampling heads and sampling lines will be determined as will the distribution of particles on the filters.

b) Dispersion of particulate radioactive materials

The concentration profiles of radioactive aerosols produced from simulated releases will be studied. Ratios of sampler concentrations to

breathed concentrations will be determined for a wide variety of conditions. It is hoped that this will provide guidelines for the positioning of personal, area and alarm samplers in real situations.

c) Ventilation pattern studies

Ventilation patterns will be studied using scale models and computer models suitable methods for visualising ventilation flows in plant and in models will be investigated. This should enable ventilation flows patterns and concentration gradients from possible releases to be optimised to minimise the aerosol intake by plant personnel.

The work in the three areas will interact to a considerable extent both as regards the facilities required and the use of information from one to aid another.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. D.H. Peirson
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Contract no.: BIO-B-333-81-UK

General subject of the contract:

Environmental studies of artificial radioactivity in soil, plants and the sea-air interface.

Description of research work:

1. Radionuclides in arable soils and crops :

a) Establishment of current levels and uptake factors.It is proposed to determine current levels of radionuclides from nuclear weapon fallout and their uptake by wheat, barley and potatoes throughout Gt. Britain, and to study geographical variations. The objective is to obtain field data which will permit more accurate assessment of the transfer of radioactive materials to man. Uptake factors and normalised specific concentration factors would be established. Retention of the radionuclides by arable soils, would be compared with results already available for undisturbed land. At some sites, current deposition would be measured by the continuous collection of rainwater samples. In general, plutonium isotopes, would be measured in all samples. The sampling sites would provide a network available to assess the impact of any future accumulations of radionuclides from atmospheric fallout. To complement the above programme, laboratory-type experiments would be performed to examine the influence of fertilier application on the uptake of plutonium and Am-241 by cereals and vegetables from soils containing relatively high levels.

b) Behaviour in soils.Differences in migration of radionuclides down the soil profile would be examined at grassland and woodland sites selected on the basis of contrasts in rainfall and radionuclide retention established from previous measurements. Profiles to at least 0.5 m would be determined. Organic (peat) soils would be included in the study. In mineral soils, the association of radionuclides with soil fractions (clay, silt and sand) would be measured and related to migration.

c) Biological effects on soils.It is proposed to make a pilot study of the response of major biochemical processes in grassland and woodland soils to high concentrations of Pu isotopes and Am-241. The radiation and/or chemical effects would be considered. Disruption of soil nitrification, denitrification and respiration processes would be examined for surface soil layers that are high in organic matter and microbial activity, which are most liable to contamination in cases of accidental releases of radioactivity. Assessment of damage to soil microorganisms can be made by studying the overall biochemical processes for which they are responsible, as has been demonstrated in previous work.

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Continuation contract no.: BIO-B-333-81-UK

2. Radionuclide enrichment of the sea-surface microlayer and transfer to the atmospheric aerosol.

Evidence is accumulating that certain elements and compounds from natural and artificial sources, which are present in trace amounts in seawater, can be enriched (perhaps by orders of magnitude) in a microlayer on the sea surface. Such enrichments may have significant consequences for their concentrations in the atmospheric aerosol above the sea and hence on the amounts transported by the wind. The present programme is concerned with studying this effect for certain radionuclides. The objects of the programme are to quantify the transfer coefficients of some radionuclides between bulk seawater and the surface microlayer, and between the microlayer and the aerosol. In order to do this, various sources and transfer mechanisms will be studied in the field and laboratory.

Following earlier work on the enrichment of stable trace elements in the microlayer, field experiments will be undertaken at a number of off-shore coastal regions to collect microlayer and bulk seawater samples for subsequent analysis for plutonium isotopes and Am-241. The microlayer sampling technique used will be a development of the earlier method, which was the collection of droplets ejected from bursting bubbles. Some stable trace elements will also be determined to seek correlations with radionuclides, and the effects of particulate and organic material content of seawater will be studied. Plutonium valency state and seawater surface tension will also be considered. The object is to demonstrate the existence, the size and the extent of the effect and to indicate the nature of the source material. The field studies will be supplemented by laboratory studies to provide more information on transfer mechanisms. The effects of bubbles passing through seawater samples containing radionuclides (including short-lived isotopes) will be investigated, together with the droplets ejected from bursting bubbles. A special wind tunnel, designed to study resuspension effects, will be used with seawater in the floor to study resuspended spray produced by wind over the water surface. The spray gross concentration and droplet particle size will be examined as a function of wind speed. Tracer materials will be used to measure enrichments in airborne spray. Other mechanisms and methods of measurements for the transfer of radioactive materials from sea to land may be investigated if the measurements described above show that they may be of significance.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-435-81-F

General subject of the contract:

Chelation of radioelements (plutonium - 239 and - 237) in the marine environment - Roles of microorganisms and various natural and bioorganic degradation compounds.

Description of research work:

Previous studies showed that the transfer of radioelements in the environment was closely linked with their physico-chemical behaviour. This depends on a certain number of factors, in particular organic ligands of natural or artificial origin. The source of natural organic ligands are microorganisms, their metabolic products and any degradation or excretion product from living organisms. It is assumed that radionuclide resolubilization phenomena exist in the upper layer of the sediments. This problem has been discussed, but the mechanisms have not been completely clarified. This phenomenon has been illustrated in agricultural soil, the fixed plutonium being resolubilized by the microorganisms. In collaboration with CEN/SCK - Belgium (Prof. O. Vanderborght), the JRC - Ispra (Dr. C.N. Murray) and the Laboratory of Radioecology in La Hague, it is planned to study the distribution of plutonium over the subcellular fractions of a eucaryotic system with the aid of biochemical methods: gradient separation of sucrose by ultracentrifugation, and determination of the mass of the compounds obtained. This will give a first insight into the respective affinities of the radionuclide for these subcellular compounds. The same techniques can be applied to the subfractions of a microorganism after sonification and to its metabolites. Following the studies carried out previously in conjunction with La Hague it is known that there are changes in the physico-chemical state (e.g. valency) of cobalt-60 and, in particular, chromium-50 after a certain contact time in seawater. These modifications falsify the experimental results. The rate of this change of the physico-chemical status depends on the presence of sediments and there is reason to believe that the activity of the microorganisms is being involved. Bioorganic compounds of microorganic or eucaryotic origin (multicellular organisms) can exist in soluble or colloidal form. The distribution of plutonium between these phases as a function of the contact time is an interesting aspect. On the other hand, there is reason to believe that the radionuclides are transferred by means of the interstitial water of the sediment. In seawater containing sediments, microorganic flora and plutonium it is intended to study the physico-chemical status of plutonium (degree of oxidation, formation of complexes, distribution between soluble and colloidal phases, etc.) as a function of the evolution of the microorganic activity in the sediment and as a function of time. In this context, it will be

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necessary to take into account the contribution of adsorption processes on the cellular membranes.
Microorganic and bioorganic activity will be monitored by measuring the ATP concentration. The following biological parameters will be studied : nitrites and nitrates, phosphates, sulphur, oxygen, calcium, magnesium, salinity, humic and fulvic acids. The determination of ATP by a photon emitting enzymatic system requires equipment available in the laboratory. It is a very accurate method which has proved its reliability in bacterial biochemistry. Isolation of the complexes will be by preparative and analytical ultracentrifugation (coefficients of sedimentation and diffusion). Their identification can be improved by high and low pressure chromatography and electrophoresis if necessary.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr.H. Smith
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Contract no.: BIO-B-336-81-UK

General subject of the contract:

Experimental studies of environmental factors influencing the uptake of radionuclides by man.

Description of research work·

A study will be made of the uptake of radionuclides into foodstuffs grown in soil contaminated as a result of liquid waste discharges from the nuclear fuel processing industry at Windscale. The soil-to-plant transfer factors which are being derived from current work are of value in modelling studies whose objective is to predict radiation exposures to the population. The contaminated soil present in estuaries near Windscale provides a unique opportunity for obtaining transfer factors for foodstuffs grown under English climatic conditions.

During 1979 estuarine sediments and more fertile soils from the Windscale region were brought to the Chilton laboratories, blended and leached with water to reduce the salt content. The soil and sediment mixture was then used to grow potatoes in tubs outdoors. Measurements are in progress to obtain the uptake of Sr-90, Cs-137, Ru-106, Rh-106, Pu-238, Pu-239 + Pu-240 and Am-241 into the potatoes. It is proposed to continue this work with potatoes and a selection of important agricultural crops. Factors to be investigated will be:

a) Changes in the availability of the radionuclides over successive seasons.
b) Variation of the transfer factors with overall concentration of the various radionuclides.
c) The influence of fertilizers on availability.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-432-81-NL

General subject of the contract:

Simulation of tritium transfer in the environment; study of organic tritium transfer in the aquatic environment, the soil-plant and the plant-mammal system.

Description of research work:

The proposed study contains four research projects intended to provide maximum comprehension of the tritium cycle in the environment. Given the complexity of the tritium cycle, the most rational approach likely to lead to an overall understanding rather than partial incomplete knowledge is to combine both modelling and experimentation. This approach assumes that the model is initially at a slightly more advanced stage than the experimental work and that a continual feedback between research workers engaged in designing models and those engaged in field and laboratory experiment is set up. The four interrelated projects involve the modelling of the behaviour of tritium and an experimental study of the transfer of organic tritium in the aquatic environment, the soil/plant system and the plant/mammal system. These projects might possibly be completed by contributions from teams working on the conversion into organic form of tritium in the soil and on tritium exchanges between the soil and the atmosphere.

1. Modelling of the behaviour of tritium. Description of this research programme is given under contract number BIO-B-340-81-F.
2. Behaviour of tritium in different chemical forms in the freshwater environment. Description of this research programme is given under contract number BIO-B-431-81-B.
3. Behaviour of tritium in the soil-plant system. Description of this research programme is given under contract number BIO-B-431-81-B.
4. Behaviour of tritium in mammals. Existing data essentially concern the turnover of tritiated water. Little data is available on the assimilation of tritiated organic matter and on subsequent developments in living organisms. Tritium may enter the animal in the form of tritiated organic materials. These materials are decomposed into small molecules by intestinal bacteria and other organic molecules are formed. Several categories of organic material may be distinguished hypothetically according to their molecular weight and turnover rate. These hypotheses must now be tested by feeding animals with various types of tritiated substances and monitoring the tritium turnover.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-B-329-81-B

General subject of the contract:

Bioavailability of actinides in selected freshwater estuarine and seawater species and the related effects of environmental factors on the modeling of their behaviour.

Description of research work:

From results furnished from the development of methodologies for the assessment of the risk associated with the accidental or controlled release of radioactivity to the environment (e.g. fallout, storage in geological formation, low-level liquid disposal), a number of important research areas have been high-lighted as requiring special attention; these are generally related to the very long-lived isotopes and specially to the actinides. One of the major areas that require more extensive study is the bioavailability of actinides in aquatic systems in relation to environmental factors that can cause changes in their chemical forms. Although data is accruing on the distribution of actinides in the environment, little is known about the mechanisms and processes that control its behaviour towards biota in fresh, estuarine and coastal waters, even less is known about its possible low-level, long-term effects. A joint programme has been developed to investigate systematically actinide behaviour in different types of aquatic systems. A brief outline of such programme is as follows:

1. Freshwater systems. The main purpose of this part is to provide biological data for the long-term previsions and the modeling of the transfer of alpha-wastes (mainly americium and also curium) in freshwater ecosystems as part of the biosphere. The general context is given by the research, under controlled conditions, on (1) the biological absorption directly from the water; (2) adsorption on suspended matter and complexation as factors that change the biological availability; (3) the uptake and transfer in successive trophic levels. In this context, we propose to investigate in more detail the difference of entrance in the biological cycle, induced by different environmental factors such as acidity, and interspecies variability and physiological status. The species used will be species living in closed freshwater systems and in river systems in important numbers in as far as they can become adapted to laboratory requirements e.g. Blicca sp., Alburnus sp., Lymnaea sp., Dreissenia sp., Polychaetae, Corixidae, Euglena sp., Elodea sp.

Emphasis will be laid on the value of indicator species in the different freshwater environments, value measured by the integrative and accumulative abilities, the ecological importance and range, the possibility to use the species as a "model" for the understanding of the physiology involved in concentration and elimination processes

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of the scarcely studies transuranics such as Am and Cm. It will also be assessed to contruct some simple bentic model communities. 2. Estuarine and coastal systems. This part of the research will emphasize an experimental laboratory programme, studying the behaviour and effects of transuranium nuclides in estuarine and coastal waters, and in certain invertebrates. This is being undertaken with the following aims: (1). Development of methods for the determination of physical-chemical states of actinides in experimental media, (2). Accumulation and retention in relation to environmental factors; (3). Studies of alpha radiation effects; (4). Estimation of absorbed doses to the species under investigation.

3. Characterization of the chemical forms of actinides in aquatic sytems. As a part of an on-going project within the JRC Nuclear Waste Management programme "Interaction of actinides with the environment", methods are being developed to study actinide chemical speciation at environmental concentrations. These methods are being applied to various studies, migration in sub-soils, bioavailability, complex formation etc.

4. Development of mathematical Methods of the mechanisms controlling bioavailability, based on the results of the laboratory studies. In order to develop better risk assessment methodologies, a clearer understanding of the mechanisms of actinide transfer in biota are required. The present proposed study will help to furnish laboratory data that could form the basis for mathematical models describing these processes. These models are essential if more realistic assessments are to be made of the behaviour of actinides in different compartments of the environment.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-B-468-81-F

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General subject of the contract:

Promotion of research and exchange of information in radioecology..

Description of research work:

The programme aims to provide support for exchange, information, standardization and specific research on Radioecology, with a view to protect man from the harmful effects of radionuclides.

1. Intercomparison and harmonization of methodologies. A continuous feedback between scientists engaged in mathematical modeling and those carrying out field studies could greatly improve the yield of research in radioecology.

a) Terrestrial environment

The transfer coefficient which is most subject to variations among different climatic and environmental conditions as encountered in the different countries is that between soil and plants.

Standardized experimental conditions (type of contamination, species of plants, description of soil, times of season) will be defined and radioisotopes most relevant for human exposure will be chosen to determine soil-plant transfer coefficients. Such conditions will also be applied to determinations on soil contaminated for some time in order to obtain information on the long-term behaviour of radioactive contamination.

b) Marine environment

Long-distance transport in sediments plays an important role for the spread of radioisotopes released from reprocessing plants (e.g. in the Eastern Atlantic, the Northland Baltic Sea). Representatives of the countries concerned (including from the Northern Countries) will define conditions of information exchange, sampling (time, character of sediments) and modelisation.

2. An inventory of present means and ongoing research as well as of future objectives of research on Radioecology in the countries where nuclear power is developed, will be undertaken. A synopsis of "Present status and objectives of Radioecology" will be prepared. Serving as ready reference for future projects to the cooperating institutes and interested organizations.

3. Training and exchange of scientists. Short-term visits to laboratories where specialized methods in radioecological research are available, will be organized for young scientists.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s): | Contract no.: BIO-C-363-81-UK

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General subject of the contract:

Molecular processes involved in radiation damage and protection at the cellular and sub - cellular level.

Description of research work:

The programme is divided into three projects which, however, are all interlinked. Together the three projects are designed to exploit fast-response techniques as aids to the understanding of the molecular processes underlying cellular radiobiological response and its modification by protective and sensitizing agents.

The technique of pulse radiolysis with optical and conductivity detection systems will be used to follow the reactions of radiation induced radicals. The time resolution of the apparatus is such that the time course of reactions occurring within a few microseconds can be followed directly. Radicals derived from DNA, free bases and ribose moieties will be produced and their subsequent reactions assessed quantitatively. The influence of agents known to affect the radiation response of cells, and in particular radioprotective agents, will be studied.

Damage to DNA and its repair will be studied by a variety of techniques. Alkaline sucrose-gradient sedimentation and controlled unwinding followed by hydroxy-apatite chromatography methods will be used to assess the degree of damage to the DNA of irradiated cells. Other assays for double strand breaks and enzyme labile sites will be developed and used to build up a picture of the distribution of the types of DNA damage and the way it is affected by dose-modifying agents. Mammalian cells and specific E. coli mutants of various radiosensitivities will be used.

The technique of rapid-lysis will be widely used in this project since it has already been shown to be capable of resolving the various stages of DNA repair in E. coli. In this technique cells are irradiated by a short (few milliseconds) burst of electrons from a linear accelerator and then at a chosen time the cells are lysed by the rapid addition of alkali and the DNA subsequently assayed. The time resolution of 0.2 sec is adequate and neutral cell-inactivating methods are being developed so that alkali labile sites and double strand breaks can be assessed separately.

The rapid-mix technique in which cellular suspensions are mixed with solutions of dose-modifying agents at controlled times before and after irradiation with a pulsed electron beam will play a major part in the cellular inactivation studies. The rapid mixing techniques provide valuable information on the role of fast free radical radiation chemical processes in cellular radiation biology, on multiple

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Continuation contract no.: BIO-C-363-81-UK

components of radiation damage and its modification by protective and sensitizing agents. Suitable apparatus with a time resolution of 5 milliseconds is available and established mammalian and bacterial cell lines corresponding to those used for the DNA work will be chosen. Using a cell survival assay the time scale and mechanism of cellular radioprotection by different classes of chemical compounds will be studied. Complementary studies will be carried out on the mechanism of radiation sensitization by oxygen and other classes of radiosensitizers. This is essential because of the interaction between mechanism of radiation protection and sensitization.

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Head(s) of research teams(s): | Contract no.: BIO-C-349-81-F

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General subject of the contract:

Lethal effects induced by the transmutation of P-32, P-33, Cu-64 and I-125 incorporated in DNA molecules.

Description of research work:

When a radioactive phosphorus atom $P^{\pm}$ (P-32 or P-33) decays (β^- emission), an ionized sulfur atom appears : $P^{\pm} - S^{+} + \beta^-$. In DNA, phosphorus is found as phosphate. After a decay inside DNA, various rearrangements which accompany the replacement of a pentavalent phosphorus atom by an hexavalent sulfur must be considered :
a) reorganization of the initial phosphate (PO_4H_3) into sulfate (SO_4H_2)
b) neutralization of the sulfur charge
c) elimination of the excedent hydrogen atom
d) dissipation of the excitation energy
e) dissipation of the recoil energy.
In double stranded DNA, these events will produce, with a probability "$\mathcal{E}$", a break in each chain via two distinct mechanisms : a break of a sulfo-diester bond (3' or 5') via processes a) and e), with a probability $\mathcal{E}_{sb}$, and a break in the opposite chain, via processes b) to d), with a probability $\mathcal{E}_{pb}$. Therefore, a double strand break will have the probability : $\mathcal{E}_{DSB} = \mathcal{E}_{sb} \times \mathcal{E}_{pb}$.
$\mathcal{E}_{DSB}$ and $\mathcal{E}_{sb}$ can be measured in experiments on the lethal effect of radioactive phosphorus incorporated into bacteriophage where a double- or a single- strand break is a lethal event. This is the case for phage T4, which has double stranded DNA, and for which a double strand break is a lethal hit, and for phage S13, which has single stranded DNA, and for which a single strand break is a lethal event.
Since P-32 and P-33 decays differ in the energy of the β^- particle emitted, the S-32 and S-33 atoms also differ in their recoil energy. And since S-32 and S-33 have no other differencies, all the above mentioned processes (a to d) are the same. Consequently, after P-32 and P-33 decays, $\mathcal{E}_{sb}$ values differ while $\mathcal{E}_{pb}$ values are equal. It therefore becomes possible to calculate, for P-33, the probability, $\mathcal{E}_{sb}$, when the probabilities $\mathcal{E}_{DSB}$ and $\mathcal{E}_{sb}$ for P-32and only the probability $\mathcal{E}_{DSB}$ for P-33 were known ; the probability $\mathcal{E}_{pb}$ is calculated from experiments with P-32. However, if, for P-33, the experimental and the calculated $\mathcal{E}_{sb}$ values are compared, the first is twice the second. This result could mean that in a phage like T4, with double stranded DNA, the number of single breaks induced by a P-33 decay is one half

the number of breaks occurring in a phage like S13, which has single stranded DNA. To test this assumption that single-stranded DNA could be more fragile than double stranded DNA, the phages T4 and S13 will be labelled with P-33 or P-32. The number of single strand breaks induced by the decay of one or the other radionuclide will be determined by sucrose gradient. In the case of P-32, no differences between phage T4 and S13 are expected, because when added to reorganization of phosphate into a sulfate molecule (process "a" above), the recoil energy of S-32 is high enough to induce a strand break with an efficiency $\mathcal{E}_{sb} = 1$. Conversly, in the case of P-33, when only the process "a" exists, a single strand break will be induced in the case of T4, with an $\mathcal{E}_{sb}$ probability of about 0.35, but with an $\mathcal{E}_{sb}$ probability of about 0.70 in the case of S13.

As process "a" alone (case of P-33) is less abrupt than processes "a" and "e" together (case of P-32), the local modifications induced by the transmutation of a phosphorus atom into a sulfur atom could perhaps reveal, in addition to the single strand break, some further modifications. These will be investigated using techniques which can detect conformational variations.

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Head(s) of research teams(s):
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Contract no.: BIO-C-361-81-UK

General subject of the contract:

Radiation damage to cellular membranes at dose levels encountered in industrial and clinical use of radiation.

Description of research work:

The research programme presented here is aimed at elucidating low dose radiation effects occurring in membranes and in membrane complexes associated with DNA.

It has been strongly implied that radiation has an effect on cellular structures other than the nucleic acids. More recent work has explicitly identified changes in membrane composition in bacterial and mammalian cells with changes in radiation sensitivity.

The main hypothesis to be investigated is "that radiation induced changes at the membrane level are a significant factor in cell death or malfunction". It is probable that changes in the DNA co-operate with the membrane changes. Several phenomena that are well established in radiobiology will be investigated bearing in mind the possible involvement of membrane.

1) Sub-lethal-damage repair
2) Thermal enhancement
3) The oxygen effect.

1) Sub-lethal damage is observed when irradiated cells recover between two or more doses of radiation. Is this recovery period a time for readjustment of membrane structure ?

2) Thermal enhancement. A preliminary heating treatment can sensitize cells to the effects of radiation. Is this due to a rearrangement, perhaps temporary, of membrane or complexes which alter radiation ?

3) In general cells are more sensitive in the presence of oxygen. Are there more or different changes in membrane structures when cells are irradiated in the presence of oxygen ?

The main measurements to be made to detect radiation induced changes are the composition and metabolism of membrane lipids in particular fatty acids, direct measurements of membrane fluidity by fluorescence polarisation; membrane bound enzyme activities and the interaction between membrane changes and DNA activity.

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Head(s) of research teams(s):

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Contract no.: BIO-E-359-81-B

General subject of the contract:

Effects of irradiation on the stability and expression of genetic information.

Description of research work:

Molecular biology has had continuous and crucial impact on many aspects of radiobiology : the "discovery" of DNA led to the identification of the major cell target for harmful radiation effects; the development of bacterial and phage genetics enabled the identification of DNA repair mechanisms in microorganisms and, a few years later in higher organism; this in turn opened the way to the discovery of their failure in several human radiation-sensitive and cancer-prone diseases. The study of the molecular mechanisms governing the early steps in the chain of events ending with the appearence of biological effects is of crucial importance in understanding the ultimate effects of ionizing radiations on living systems. The introduction of new physicochemical techniques, heavily grounded on computer-assisted analysis of data, now renders possible the detection and the identification of free radical intermediates in highly complex systems, such as nucleic acids and nucleoproteins. In addition, the use of suitable non toxic additives capable of interacting with these unstable intermediates in order to deflect the energy degradation pathways from nucleic acids to irrelevant targets is currently under investigation. An important breakthrough in this domain is to be expected in the next few years. The discovery of induced SOS mutagenic repair in this laboratory prompts us to continue these investigations in mammalian and human cells by using singlestranded viral probes, somatic mutagenesis being implicated in tumor initiation. In addition an original enzymatic method for measuring the somatic mutation load of a human being will be developed. New bacterial strains developed in this laboratory and capable of distinguishing genetic and epigenetic changes caused by environmental agents will be used for a quantitative study of the genetic and epigenetic effects of ionizing radiations, and adapted as biological dosimeter for low doses of ionizing radiation. Radiation induced (mis) recombination on the other hand may be implicated in chromosomal rearrangements and aberrations and in tumor promotion. These events, just like mutations, may be of inducible nature and therefore should be amenable to prevention by appropriate inhibitors like antipain, an inhibitor of X ray induced oncogenesis. The study of recombination and gene transposition in this department is also a prerequisite for understanding control mechanisms of gene expression and cell differentiation during which specific chromosomal rearrangements are known to occur in several systems. Cell hybrids will

be used to study the control of radiosensivity of mammalian cells and of oncogenic transformations; special attention will be devoted to heterozygotes originating in cancer prone human families. The extent of variability of radiosensitivity in large numbers of normal individuals, cancer patients and cancer prone conditions will be determined using the micronucleus method in peripheral blood lymphocytes. A comparative cytogenetic analysis of radiosensitivity during maturation of the mouse and human oocyte will be undertaken in metaphases I and II and cytoplasmic maturation will be studied after irradiation. Low doses of X rays during cleavage of mouse embryos will enable to establish dose-stage relationships and permit observations on radiation damage and repair during the early stages of development, as well as on teratogenesis after re-implantation. Intensive studies in this laboratory have contributed to the unravelling of immunoregulatory circuits based on interlymphocytic communications. A number of studies have pointed out quite clearly that, in the immune system, suppression is dominant. Silent lymphocytes are not insignificant minorities but are kept under active repression, mainly due to radiosensitive T lymphocytes. It is therefore of utmost importance to study the possible relationships between low dose irradiation and autoimmune processes, both in rodents and in human peripheral blood lymphocytes. Furthermore, our increased knowledge of the rules of the game of the immune network allows us to attempt to construct immunocompetent allogenic radiation chimaera.

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Head(s) of research teams(s):

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Contract no.: BIO-C-343-81-D

General subject of the contract:

Radiation induced degradation of nucleobases in DNA : primary effects and structural changes.

Description of research work:

DNA is considered to be an essential target for radiation induced cell inactivation and mutagenesis. Previous studies of the primary effects of radiation on nucleic acids have demonstrated a variety of modifications, i.e. chain scissions and changes of the nucleobases which alter the molecular structure of the genome. Many data exist already on the formation of single- and double strand breaks of irradiated cells, but much less is known about the modifications of bases and base loss. This so-called "base damage", however, is considered to contribute to the biological radiation effect as well as strand breaks since it certainly also affects the function of DNA during transcription and replication.

The aim of this research program is to analyse the radiation induced base damage in DNA of living cells. Particular emphasis should be given to the interface of reactions between radicals and stable radiation products as well as to the interface between the radiation products and structural changes in DNA. These research lines should lead to a better understanding of the contribution of DNA base-damage to important biological radiation effects such as cell inactivation and mutation.

Concerning the oxygen effect in irradiated organisms, there is considerable knowledge about OER-values for various radiation effects including induced strand breaks in DNA but much less is known about the reactivity of base moieties with oxygen radicals and the consequences of the formation of peroxy and alkoxy radicals. Experiments are therefore planned to study the reactivity of these radicals with DNA moieties and the effect of additives on the nature and yield of DNA base damage. This entails re-investigation of the actual reactivity of the oxygen radicals $^{\circ}OH$ and $O_2^{\cdot}$ with DNA compounds, evaluation of the combinatory effect of these and other radicals as well as elucidation of possible interactions with biologically generated oxygen radicals.

To evaluate base degradation in DNA of irradiated organisms, several ways can be followed: (a) using sensitive analytical methods for detection of relevant radiation products, (b) measuring "endonuclease sensitive sites" using specific endonucleases or (c) analysing the base pairing ability of DNA, to detect sites which are unable to pair with the complementary base, i.e. with mismatched base pairs or regions with interrupeted hydrogen bonds. The last approach shall be followed in this study. From the results of our previous studies we conclude, that in gamma-irradiated phages local denatured regions are introduced in

addition to chain scissions and modified bases. In and around these single-stranded regions modified bases sites with lost bases may be located. These heteroduplices are detectable only in DNA of gamma-irradiated phages, but not after irradiation of DNA solutions. Apparently, two types of base damage are significant for the irradiated living organism: (a) structural changes of single base moieties and (b) heteroduplex sites, formed by a direct effect of radiation. In future studies along this line heteroduplices in irradiated cells should be analysed and their location in the genome as well as dose-effect relations should be determined.

Both of the approaches outlined in this research programme must be followed simultaneously, as the knowledge of primary events on nucleobases is necessary for the understanding of the formation of heteroduplices, i.e. whether these sites could be formed by charge migration within the stacked bases in DNA or formation of a cluster of base damages in this region.

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Head(s) of research teams(s):

Contract no.: BIO-C-342-81-D

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General subject of the contract:

Studies of the effects of ionizing radiation on DNA and its constituents.

Description of research work:

The research aims at the elucidation of dominant mechanisms by which DNA-damage is induced at a molecular level by ionizing radiation. In continuation of the research under the previous contract specific emphasis is given to both systems and techniques which allow for a comprehensive investigation of radiation-induced free radicals, their structural features, their quantitative contributions under varying experimental conditions, as well as their formation and decay reactions, respectively.

The research programme is devoted mainly to the study of free-radical mediated mechanisms of induction of DNA strand breaks subsequent to attack of water-radiolysis radicals in dilute aqueous solutions and frozen glasses, as well as their dependence of external additives like electrons or hole scavengers. It is hoped, by these studies, to be able to give definitive clues to radiation-chemists for relating the observed irradiation products to structurally and mechanistically feasable intermediates.

Strand breaks in DNA, especially double strand breaks, form a damage of specific importance with respect to inactivation of the DNA-functions. There have been extensive studies both in the field of enzymatic endgroup recognition, repair and other biochemical-biological aspects of DNA strand breaks as well in the field of radiation-chemical analysis of phosphate release in DNA and in model compounds in previous years. These investigations have yet to be amended by studies of initial mechanisms leading to strand break formation. In the system of "direct" radiation-action, specifically in single crystals, radicals located at the deoxyribose-phosphate backbone have received increasing attention over the past few years. Such studies have added to the basic knowledge about the primary mechanisms of radical formation in solid sugars and sugar phosphates but have so far been unable to provide a link to reactions occurring in solution. It is attempted in the present programme to contribute to the establishment of such links by using either solutions or systems intermediate between single crystals and liquid solutions, like frozen glasses. In doing so, and by the additional use of hydrated single crystals, the role of water in mediating free-radical reactions will be assessed.

The compounds proposed for study will be nucleotides, dinucleoside-phosphates, polynucleotides and, whenever possible,

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DNA itself (preferably in oriented form) as well as subunits like single bases combined with sugars and sugar-phophates in order to study competition reactions and radical transfers. The inherent problem of lack of spectral separation of radicals in complex DNA-constituents will be attacked by using ESR-spectroscopy at X-, K- and Q-band (10, 24 and 35 GHz) combined with single-crystal and "powder-type" ENDOR-spectroscopy as well as optical spectroscopy. In addition, the systems will be studied in an environment providing for single reactive species attacking the solute ($H^{\cdot}$, $OH^{\cdot}$ and e^{-}_{aq}) prior to subjecting them to X-irradiation. Finally, extensive use of computer-separation of spectra as well as of combined spectra-simulation (MAGNSPEC) and wavefunction calculations of radicals (INDO) will be made. These methods should provide an insight into both structural and quantitative estimates of free-radical mediated pathways leading to DNA strand breaks in a highly aquated environment.

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Head(s) of research teams(s):

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Contract no.: BIO-C-470-81-D

General subject of the contract:
Effects of ionizing radiation and chemical agents in combination at the molecular level, over the dose range encountered in clinical and industrial use.

Description of research work:

Alterations in the DNA, unrepaired or misrepaired, can have lasting consequences ranging from cell death and mutations to malignant transformation and subsequent carcinogenesis. Analysis of such damage to DNA is, therefore, the main objective of the Radiation Protection Programme at the Primary Effects level. These studies will continue but a new emphasis will be made for studies of the combined effects of chemicals and radiation. There is a vast number of chemicals present in our environment that can act as mutagens and carcinogens, however, very little is known about their effect in combination with low doses of radiation. In many instances it is an open question whether such interaction is synergistic, antagonistic or just additive. Although very little is known about possible synergistic effects the problem has assumed an increasingly important role in recent discussions of radiation protection and risk assessment. The present research programme is aimed at an elucidation of the combined effects of ionizing radiation and chemical agents known to interact with DNA. It is in line with the "Primary Effect" programme. Numerous groups within that programme work on the analysis of primary alterations produced by ionizing radiation. It is the intent of this programme to achieve a linkage between such work and the study of more complex biological systems. Therefore main emphasis will be given to the correlation of biological and physicochemical endpoints.
The biological systems to be used in this investigation are :
1) Supercircular plasmid DNA. This system is very suited for the simultaneous investigation of true single and double strand breaks as opposed to alkali labile lesions, for the investigation of base release and crosslinks. The conversion of supercircular DNA into circular or linear DNA will also be used to study the effect of chemicals added prior to irradiation.
2) Biologically active phage DNA. Since the sensitivity of most physicochemical methods is a function of the homogeneity of the DNA used as substrate, infectious phage DNA allows a correlation of physicochemical and biological endpoints with high sensitivity.
The techniques and methods to be used include DNA transformation and transfection, ultracentrifugation, gel electrophoresis, radioactive labeling, fluorescence spectroscopy, DNA unwinding method and hydroxyapatite chromatography. We also intend to adopt the improved DNA unwinding method which allows to investigate the strand breaking

efficiency of harmful chemicals and radiation at low doses. Most of the mentioned techniques are already in permanent use in this laboratory. It is one of the major features of the submitted programme that it is not restricted to sparsely ionizing radiation but also includes neutrons. This is especially important in view of the unknown dependence of synergistic effects on LET.
It is hoped to collaborate with the Cyclotron Unit at Hammersmith, London, mainly in sharing their experience with radiation sources. A collaboration with the Radiation Chemistry Group of the Max-Planck Institute, Mülheim, is already in existence for studying the chemical structure of radiation and chemically induced DNA lesions. This collaboration will be intensified under the proposed programme.
For the selection of harmful chemicals the following approach is envisioned. The major lesions induced in DNA by ionizing radiation are strand breaks, crosslinks and base alterations. Very similar damage can be produced by strand breaking, crosslinking, intercalating and alkylating agents. Chemicals of well established interaction with DNA will be used together with radiation to develop a model system which may then be extended to other compounds that are critical for environmental considerations.
It is planned to start the experimental work with intercalating agents, because these chemicals disturb the molecular structure of the DNA and may influence the transfer of excitation energy in the DNA molecule induced by ionizing radiation. Ethidium bromide and propidium bromide are considered as possible candidates. Chemicals which influence base release and strand break production will be used in the subsequent phase of experimental work.
With the biological systems and techniques already in use in the laboratory it is hoped to answer such questions as :
1. Is there synergism between chemicals and radiation ?
2. Can such interaction be modified or eliminated ?
It is felt that the results will lead to a better understanding of the combined effects of chemicals and radiation especially with respect to synergistic interactions.

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Head(s) of research teams(s):

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Contract no.: BIO-C-356-81-NL

General subject of the contract:

Investigation of the relationship between radiation-induced primary damage in DNA and the biological activity of DNA.

Description of research work:

Since about 1970 an extensive insight has been gained into the nature and yield of numerous products formed as a result of DNA irradiation mainly under conditions of prominent indirect effect. Little is known, however, about the biological significance of the majority of these chemical changes. The objective of the research programme is to gain a deeper insight into the relationship between specific radiation products in DNA and their biological significance. At the same time a study will be made of the effects of protective compounds (e.g., sulphydryl compounds, ascorbic acid) and of the oxygen sensitizer. This study will make use of the biologically active single and double stranded DNA of the bacteriophage ØX174.

One of the difficulties of establishing a relationship between radiation-chemical changes in DNA and their biological consequences, arises from the wide variety of products resulting from irradiation under indirect-effect conditions. In principle, it now seems possible to simplify the product spectrum by irradiating DNA in frozen solutions: ESR studies on nucleotide mixtures or DNA irradiated in ice have in fact shown that specific radicals are formed (mainly as a result of direct effect). Most of these radicals are located on the bases guanine and thymine, and presumably give rise to a more limited number of products than would ensue from irradiation in a liquid medium. This becomes apparent in the case of thymidine irradiated in a frozen solution (195 K): apart from some small fraction of thymine-dimers, practically all that is produced is dihydrothymine derivatives. This contrasts with the much more complicated product spectrum resulting from irradiation in solution and opens up the possibility of irradiating biologically active DNA in order to correlate, to a more specific extent than can be achieved by irradiation in water in the liquid state, the lethal or mutagenic effects with predetermined radiation products. The specificity can probably be increased still further by the addition of suitable electron donors or acceptors prior to freezing.

I. Inactivation

a. The same procedure that is followed after irradiation in water in the liquid state can be used to determine the lethal (or benign) character of a number of chemical changes (such as dihydropyrimidine

derivatives, sites from which purines or pyrimidines have been removed, direct and latent fractures) after irradiation in ice and thawing of the sample. A recent publication, in principle, also opens up the possibility of detecting whether or not purine damage is lethal. This is especially important after the irradiation of DNA in ice, where one of the damage sites has been shown to be on the guanine base. The usefulness of this method of detecting purine damage is currently being examined, and further elaboration and applicability tests will be necessary in the context of this research programme

b. Corresponding analyses of DNA can be carried out after the irradiation of complete ØX174 bacteriophages and/or of ØX DNA-histone complexes in ice or in water in the liquid state. The DNA-histone complexes are important in view of the incidence of DNA complexed with histones in eucaryotic cells.

II. Mutations

The overall inactivation of ØX 174 DNA by radiation will be caused, in part, by point mutations; in a number of these mutations sense - triplet are converted into nonsense codon or stopcodons. As a rule, a stopcodon is lethal, since it prevents the formation of complete virus particles in the normal E. coli host. However, a large number of E.coli suppressor mutants are known to exist which can recognize a nonsense codon as a sense codon, so that a ØX DNA molecule with that particular nonsense codon is indeed viable in the suppressor E. Coli. These bacteria are highly suitable for the study of point mutations in bacteriophage DNA.

a. The induction of mutations will be studied after the irradiation of ØX174 DNA in water in the liquid state (indirect effect) as well as in ice (direct effect : with the advantage of a more simplified product spectrum). In principle, this is possible by the application of experimental methods similar to those required for the work described in Section I. For the purposes of this work, it is necessary to increase the efficiency of the test for the determination of the biological activity of the ØX DNA. In the light of recent work, this appears to be feasible.

b. Another method to investigate the mutagenic nature of the DNA radiation products, starts with the DNA from well-known nonsense mutants of ØX174 DNA and measures back-mutations to (pseudo) wild type DNA. These so-called revertants can multiply in the normal E.Coli host in contrast to the nonsense mutants themselves. The mutagenic effects of ionizing radiation in complete ØX bacteriophages (under direct effect conditions) have already been studied along these lines. Since the site of the mutation of a variety of these nonsense mutants, is exactly known, this study may help to throw light on the radiation-mutagenesis mechanism.

c. Similar work can also be carried out after the irradiation of the ØX DNA in the form of DNA complexes with histones or with other DNA-binding proteins.

The influence of certain protecting agents and of O_2 will be investigated for each of the subjects discussed in Sections I and II.

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Head(s) of research teams(s):

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Contract no.: BIO-C-469-81-D

General subject of the contract:

The influence of protecting agents on radiation damage processes in biomolecules.

Description of research work:

It is planned to investigate radiation effects in biomacromolecules and corresponding molecular assemblies. Prominence shall be given to the study of crosslinking and aggregation processes, which contribute to the establishment of permanent damage effects. The investigations shall be carried out with the aid of the pulse radiolysis method in conjunction with time-resolved Rayleigh light scattering measurements. Native and denatured DNA as well as DNA histone complexes and histones shall be subjects of the investigations. Preliminary experiments with native DNA revealed that crosslinking and double strand breakage can be clearly separated. A careful extension of this work shall be especially devoted to the influence of protective agents on the observed damage effects. Typical protectors of the sulfhydryl and amine type shall be tested.

Moreover, parameter studies shall comprise the influence of ionic strength and initial molecular weight of the DNA. In a similar way, histones and histone-DNA complexes shall be studied. Parallel to the pulse radiolysis studies, stationary irradiations shall be carried out. Thereby conventional techniques, such as gel electrophoresis, shall be used for the characterization of the products and the determination of radiation-chemical yields.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-341-81-D

General subject of the contract:

Chemical studies of chain breaks in DNA induced by gamma - irradiation.

Description of research work:

A. Studies on model systems

Whereas in deoxygenated aqueous solutions DNA strand breaks are mainly produced by the scission of a phosphoric acid ester bond, breaking of a C-C bond plays a considerable role in oxygenated solution. Peroxyl radicals are intermediates in these reactions. Our present knowledge of the fate of peroxyl radicals is limited. Therefore, it appears necessary to study such reactions more extensively using model compounds.

Whereas oxygen is known to fix radiation damage there is a number of compounds which show a protective effect, most prominently those containing sulfhydryl groups. It is not yet clear, how these compounds interfere with the strand breaking process. Nucleotides appear suitable substrates to study these processes on the model level.

B. Studies on DNA

The processes that lead to DNA strand breaks if the DNA is irradiated in aqueous solution has been largely elucidated in the period of the last five years contract. It has been argued that in the living cell not only the indirect effect as studied with aqueous solutions but also the direct effect should play an important role. The latter can be studied by irradiating dry or moist DNA. Up to present no chemical investigation with respect to the primary processes leading to strand breaks in dry or moist DNA is available. In order to evaluate the reactions occuring in cells (see C) the dry and moist DNA system has to be investigated as a model.

C. Studies on cells.

The ultimate goal is to understand the reactions leading to DNA strand breaks in living cells. Two systems are envisaged. In the first system, E. coli bacteria, the DNA is not connected with nucleohistones and there the situation might resemble somewhat that already studied with DNA in aqueous solution. In the second system, nuclei isolated from chicken erythrocytes, the DNA is connected with the nucleohistones as in other eukariotic cells. The advantage of working with isolated nuclei instead of with whole cells will be the absence of the cytoplasma which would additionally hamper the isolation of fragments from the damaged DNA.

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Head(s) of research teams(s):

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Contract no.: BIO-C-362-81-UK

General subject of the contract:

Application of spectroscopic techniques especially e.s.r. to the study of radiation effects in DNA and DNA-protein complexes.

Description of research work:

The overall aim will be to study the ways in which suitably selected additives directly modify the effects of ionizing radiation on DNA. It is fully realised that many potential additives could prove to be too toxic to be of any used in living systems but, at present, the aim is to understand what happens at a molecular level in properly designed systems, rather than to search for viable compounds. It is also realised that many materials which have a moderating effect in vivo act by a variety of mechanisms which may often be very indirect. Nevertheless, studies on direct effects of additives may well point the way towards effective moderation of radiation processes and should certainly be mechanistically informative. At least four systems involving DNA should be probed : a) Frozen aqueous DNA +/- additives b) Frozen aqueous DNA-histone complexes +/- additives c) Glassy aqueous DNA +/- additives d) Glassy aqueous DNA-histone complexes +/- additives. It may well be necessary to use simplified model systems to re-inforce results and interpretations, but such studies will be subsidiary to work on DNA. This project will concentrate on (c) and (d), whilst Prof. Bertinchamps' project will concentrate on (a) and (b). Additives will cover a wide range of compounds expected to act as radiation moderators. They will also include materials that on photolysis will give various species such as e^- and $^{\cdot}OH$ radicals so that their effects can be studied in isolation.

The distinction will be made between additives, such as certain cations, that bind directly to DNA or its complexes, and those which act simply by being in the aqueous systems. For example, certain histones themselves may contain RS-SR units. These readily add electrons to give $RS \overset{\cdot}{-} SR^-$ radicals which can be characterised by e.s.r. and by optical spectroscopy. Since there is a marked shift in g from the free-spin region, these radicals can be estimated quantitatively despite the presence of several other species. Thus, in principle at least, it may be able to follow the suppression of thymine anions when RS-SR groups are present.

In the work on electron-capture by aqueous glasses containing metalloproteins it was established that this can be an extremely efficient process in aqueous glassy systems. Electron capture can be followed quantitatively by measuring loss of e.s.r. signals on capture by paramagnetic systems (Fe (III), Cu (II), etc.), or gain of signals when the substrates are diamagnetic (Mo (VI), Fe_2S_2 units, etc.). In

most cases, the signals are widely removed from the g=2 region and hence are readily monitored, and will not interfere with monitoring of DNA radicals in this region. These selected proteins may interact directly with DNA samples or may be part of the bulk aqueous sustems. In order to probe electon-capture processes in isolation, photo-ionizable additives will be included in the systems, and the e.s.r. spectra will be monitored after exposure to UV light.
Hole-capture is not so well established and may be more difficult to accomplish. For glassy systems the major e^--loss species, H_2O^+, very rapidly becomes $^{\cdot}OH$, and hence $^{\cdot}OH$ radical scavengers will be needed. Prevention of direct electron-loss may possibly be accomplished using additives that bind directly to DNA. For example, electron-loss from RSH to give $R^{\cdot}SH^+$ and thence $RS^{\cdot}$ might be effective. It has been shown that $RS^{\cdot}$ radicals give rise to e.s.r. features that are again well removed from the free-spin region, but are strongly dependent on environment. Hence it is hoped that it can be established whether or not RSH compounds can act as electron-donors in DNA systems. Other potentially potent electron-donor additives to be studied will include electron-rich transition metal complexes (Ag (I), Cu (I), Co (II), etc.), and the reduced forms of metallo-enzymes.
Attack by $^{\cdot}OH$ radicals should occur when irradiated glassy aqueous systems are annealed. Again, in order, hopefully, to probe this step in isolation, systems containing H_2O_2 will be photolysed, either using glassy solids or using fluid flow systems, possibly in conjuction with rapid freeze techniques. The effect of additives that may inhibit attack of $^{\cdot}OH$ radicals on DNA will then be probed, including species which may bind directly to DNA or its complexes. Whenever additives are expected to give rise to organic radicals with features in the free-spin region, we will use perdeuterated material, in order to avoid obscuring the features from DNA radicals. Thus for example, $(CH_3)_3COH$ or glycol, added to scavenge $^{\cdot}OH$ (and also possibly to encourage glass-formation), will be used in their perdeuterated forms. These experiments are open-ended in that there is a very large range of possibly significant additives to call upon. As their separate effects begin to be unravelled, attention will be given to the use of complementary pair of additives in the expectation that these may act in a co-operative fashion.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-350-81-F

General subject of the contract:

Study of DNA injury caused by ionizing radiation.

Description of research work:

Modifications of the chemical structure of DNA caused by ionizing radiation are responsible for most of the radiobiological effects. These modifications are the result of a long chain of free-radical reactions often involving the primary species of the radiolysis of water : H°, OH° and e^-_{aq}. The Radiobiochemical Laboratory of CEN, Grenoble, in collaboration with the European laboratories of the Primary Effects Group, will analyse these transformations of free radicals which lead to final products. In past years, it has established the nature of the principal final products of the degradation of natural nucleosides, with the exception of desoxy-2' guanosine; recent technological advances now make this study quite feasible.

Dinucleotides and oligo desoxyribonucleotides of known sequence, exhibiting one precise alteration in a well-defined site obtained by chemical means or by irradiation, will be used to study the early effects of ionizing radiation at a molecular level. Access will thus be obtained to an appropriate system, capable of being analysed by modern chemical methods and of being utilized by current molecular biology techniques. Even with compounds as simple as the specifically modified dinucleotides, which exhibit an alteration identical to that produced under irradiation, a great deal of information will be obtained, particularly with regard to the mode of action of the enzymes involved in cell repair : phosphodiesterase, glycosidase, etc. The modified oligonucleotides will be used for preparing, where necessary, radioimmunological reagents against certain types of lesion produced by radiation and thus contribute to the development of in vivo dosage methods. For this reason, the chemistry of modified oligonucleotides will be developed and will be an important interface between biologists and chemists studying radiation effects.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-358-81-B

General subject of the contract:

The effects of ionizing radiation on the defence mechanisms against infections.

Description of research work:

Injuries of the immune system are one of the major results subsequent to irradiation. At low doses, malignant transformation has been shown to especially affect cells of the lymphoid system and to induce malignant tumours such as myeloid leukemia or multiple myeloma. At high doses, short term effects such as an important immunodepression are added to the previous carcinogenic consequences of radiation exposures. However, enhancement or suppression of the specific immune responses have been observed after irradiation. Such opposite effects are still poorly understood. Investigations are needed and, at the present time, can be undertaken as numerous subpopulations of lymphoid cells and control mechanisms of the immune response have been identified during the last few years. Their respective radiosensitivities are not known and will perhaps greatly help to understand the radiation induced immunodepression and perhaps give new possibilities to correct it. The host defence mechanisms against infections are multiple. They could be divided into non specific and specific factors. Amongst the non specific factors are the so-called normal commensal flora which play a very important role by competition with the pathogenic microorganisms. The specific factors are composed of humoral (antibodies and complement) and cellular immunity but also of lots of mechanisms which combine antibodies and cells such as macrophages, neutrophils, eosinophils... . It seems of great interest to study the radiosensitivity of the defence mechanisms against infections due to pathogenic organisms as, very often, they can cause fulminating infections. In such cases, the speed of development of the microorganisms can overtake the therapeutic effect of the chemotherapy. It is quite possible that new techniques such as monoclonal antibodies could really improve the present therapeutic possibilities. But, what is perhaps more important is the problem of the non pathogenic microorganisms which represent the great majority of the bacteria causing septicaemiae after irradiation. Their rare and occasional virulence, in normal situations, cannot be considered as a real problem in medicine. However, in irradiated patients, they can produce severe septicaemiae and so, must be studied in radiobiology. The consequences of irradiation on the defence mechanisms against infections either by pathogenic or by non or conditionally pathogenic microorganisms will be studied. The use of immunodeficient animals will allow a first screening of the mechanisms by which the "normal"

flora can invade the organisms. B lymphoid cells can be suppressed by repeated injections of xenogeneic serum anti mu heavy immunoglobulin chain. Such injections must begin at birth and be repeated every other day during the whole life of the animals. T cell depleted animals can be obtained by using genetically thymusless animals such as the rnu/rnu rats. Different techniques can diminish the macrophage activity such as injections of silica. These different models will be tested for their sensitivity to non pathogenic organisms which can generally be found in the intestinal tract, such as Echerichia coli. Yersinia enterocolitica which is strictly non pathogenic in the rat species will also be considered. By graft of cells from normal histocompatible animals, the defect due to irradiation will also be studied in order to characterize the radiosensitive protection mechanism. Such studies will perhaps improve the present knowledge of the occasional pathogenicity of microorganisms from the so-called "normal intestinal flora" and offer new possibilities to cure irradiated patients suffering from septicaemiae.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-351-81-F

General subject of the contract:

Molecular mechanisms of X-ray and UV induced cataracts : study of the DNA stability and genetic expression of lens proteins using tissue culture methods.

Description of research work:

The lens is a suitable model for studying the effects of ionizing radiations on a pure population of cells, different from fibroblasts, on the phenomenon of terminal differentiation and on post mitotic cells. In 1903 Tribondeau demonstrated the ability of X-rays to induce experimental cataracts in animals. Despite the great number of publications, much emphasis was put on the late effects of X-rays on cataractogenesis and not on the early events. As a matter of fact the lens is a very simple organ well suited to a molecular and cellular approach of the initial steps. Cells from the equatorial region differentiate progressively in fibers : the nucleus becomes pyknotic before disappearing totally in the mature fiber. This cell will enlarge enormously and be filled up with newly synthesized crystallin proteins. These processes occur during the whole lifespan and can be reproduced in vitro in different systems.

1. terminal differentiation occurs spontaneously in culture in the chick embryo.
2. the central epithelium in adult lens is composed of a stable, amitotic cell population maintained in Go in culture for a few hours by incubating the whole lens (human, chicken, rats).

It is proposed that these models be used to analyse the genomic stability and the expression of lens specific proteins under the comparitive effect of radiations.

I. Effects of ionizing radiations on chick embryo epithelium.

I.a) At the enzymatic level : are all the DNA synthesizing enzymes present and functional ?

It was described that the repair system was either lacking or impaired in function of the embryonic stages. It is proposed to record the DNA polymerase alpha, beta, gamma, the thymidine kinase, and the ligase I and II activities at these stages in different culture conditions (after 3 days in vitro). DNA polymerase alpha is known to play a role in replication while DNA polymerase beta could be a repair enzyme. Could enzyme beta, ligase I or II be lacking at one of these stages ?

I.b) Correlation between broken DNA either normally or after irradiation and specific protein synthesis.

It was shown that cycloheximide can modulate the DNA breakage. This delay does not seem related with delta crystalling synthesis (another specific marker of differentiation in chick lens) : for a concentration of 0.01 µg/ml, 72 hr which delays the DNA breakage, the

delta crystallin specific activity is identical either in presence or in absence of the antibiotic. In this system, delta crystallin is the main synthesized protein. It is expected that the synthesis of either the other crystallins (alpha, beta) or minor proteins could be affected. It is interesting to study their pattern and synthesis in the following conditions.

a) when the DNA accumulates breaks progressively as the physiological process of terminal differentiation appears.
b) when the DNA is broken artificially by ionizing radiations either in the whole lens or in the epithelium separated from the fibers.
c) when the normal DNA breakage is delayed by either cycloheximide or by a medium lacking of tryptophan (known conditions to show in vivo cataracts with a delay in the nuclear pyknosis).

I.c) Relation between the differentiation rate and the ionizing radiation sensibility.

It is known that lenses of young rats are much more prone to radiations than those of older animals. It is possible to modulate the differentiation rate either by delaying DNA degradation (cycloheximide, absence of tryptophan) or by increasing the rate of differentiation (following Beebe's method). It was indeed discovered that in the vitreous, a factor exists which increases the elongation of the central epithelium chick lens cells (6 day embryonic stage).

This laboratory described the presence of a similar factor in the retina that allows the elongation of lens epithelial cells in culture. It would be interesting to study the possible relationship of the differentiation rate, since it is know how to modulate this rate, and the sensibility to ionizing radiation.

II. Effects of ionizing and UV radiations on the lens central epithelium from different species.

The stability of Go cells located in the central zone of the adult lens epithelium is a well established phenomenon. It will be very interesting to study these epithelial cells in culture irradiated with different doses of X-rays and UV and to analyse DNA repair and to compare it as a function of the age of the donor. The synergism between these two radiations may conduct the lens cells to accumulate damages giving rise to earlier cataracts. This shall be done by 1) autoradiography, 2) DNA sedimentation in alkaline sucrose gradient, 3) specific enzymic action on single stranded DNA (nuclease S_1), 4) immunocytochemistry. Thus a possible correlation between cell aging and either UV or X ray sensibility could be detected. Some preliminary experiments show that there exist in the central epithelium more sensitive regions to these irradiations.

The use of these <u>in vitro</u> systems seems to be of interest to evaluate the radiation effects at short and long term on one mostly sensitive organ : the eye.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-C-354-81-I

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General subject of the contract:

Radiation damage and recovery of the immune system.

Description of research work:

The aim of this research program is the early identification of radiation damage to the immune system and the continuous evaluation of the extent of the lesions and of their consequences.
Studies of the effects of irradiation on the immune system are very relevant to radiation protection if dealing with short-term effects likely to impair defence mechanisms against infections and with late effects altering the surveillance mechanisms against tumour appearance and development. In the past, most of the observed functional alterations in irradiated animals have been attribued to elimination of immunologically competent cells.
Advances in cellular immunology have identified three main cell populations involved in immunity processes. There are two lymphocyte populations : B cells responsible for antibody production; T cells responsible for cell-mediated responses (rejection of foreign cell and tissue grafts, immunity against tumour cells) and for regulation of immune responses. Regulation was originally thought to be exerted by two T cell subpopulations with antithetic functions : helper and suppressor cells, More recently, the combination of an unique cell-surface marker and of a particular function has allowed in the mouse the identification of the following T cell sets that interact in a complex regulatory network : Lyl-marked inducer cells; Lyl, 2,3-marked regulatory cells; Ly2,3-marked killer and suppressor effector cells.
The third main cell population is represented by accessory cells, such as macrophages and reticulum cells, which interact with antigen and the two lymphocyte populations. Immune reactions are determined by signals passed among different types of cells that regulate the intensity and duration of the response after perturbation of the system by the immunogen. Communication among different cell types is also essential for preventing autoimmune diseases. The immune response results from perturbation of a homeostatically balanced system set in motion by antigenic activation of Lyl^+ inducer cells which, as sentinel cells, screen the surface of accessory cells for antigen. Lyl^+ cells can induce a variety of effector cells, such as B cells to produce antibodies, macrophages and leucocytes to participate in delayed-type hypersensitivity reactions, pre-killer T cells to become killer T cells, and, in addition to immunological actions, can induce

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osteoclasts to resorb bone and colony-forming cells to differentiate into mature erythroid elements. Once stimulated, the sentinel cells can activate a set of $Ly1,2,3^+$ cells which supplies and regulates the function of inducer $Ly1^+$ cells and suppressor $Ly2,3^+$ cells; one subset of $Ly1^+$ cells activates $Ly1,2,3^+$ cells to generate $Ly1^+$ cells that help B cells to produce antibodies, while another subset of $Ly1^+$ cells stimulates $Ly1,2,3^+$ cells to suppress $Ly1^+$ cells either directly or through the generation of $Ly2,3^+$ suppressor cells. Inhibition of $Ly1^+$ cells by this feedback suppression mechanism results in inhibition of both help and suppression of antibody production. In this balanced system, accessory cells play a key role by presenting the antigen in a form appropriate to the ultimate activation of helper or suppressor cells, depending on the genetic background of the host.
The immunoregulatory mechanisms discovered in the mouse are likely to be the same in the man as expected from the similarities of certain cell types and functions already identified in both species. Studies of immune defects in the mouse model should provide informations which could be usefully extrapolated to man. There are, indeed, spontaneous diseases in NZB and MRL mice and in man characterized by production of autoantibodies and clinical symptoms of systemic lupus erythematosus. These diseases are associated with defective communication among T cells consisting of the inability to inhibit $Ly1^+$ cells due to lack of $Ly1,2,3^+$ cells in NZB mice or to insensitivity of $Ly1^+$ cells to suppression in MRL mice. It is noticeable that total lymphoid irradiation ameliorates symptoms and survival in NZB mice.
Irradiation of the immune system is likely to produce lesions that perturb its homeostatic equilibrium early and late after radiation exposure. Early effects may reflect depletion of radiosensitive cells, while late effects may result from dysproportionate proliferation and differentiation of the recovering cell population, sets and subsets required for the maintainance of normal immune functions. The proposed investigations will be carried out in mice exposed to ionizing radiation. X-rays will be administrered either as total-body irradiation in a single dose in the low sublethal range or as partial-body irradiation in fractionated doses according to the regimen of total lymphoid irradiation. The latter treatment has been successfully used in the therapy of human lymphomas and has experimantally proved to be very useful for induction of transplantation and self tolerance with few side effects and negligeable mortality. Early and late after either radiation treatment helper activity, suppressor activity, and antigen presentation by macrophages will be evaluated by in vitro techniques suitable to dissect out the cell components of the immune system and to assess their radiation damage and recovery. Treatment of radiation-induced alterations of T cell functions will be attempted by injection of thymus epitelial factors of extractive and synthetic nature.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-360-81-B

General subject of the contract:

Thyroid irradiation : Radiobiological consequences of irradiation in cell culture systems and in humans.

Description of research work:

The thyroid is often exposed to irradiation in diagnostic tests and therapy and as occupational hazard in laboratories and nuclear plants. The consequences of thyroid irradiation include functional disturbances, hypothyroidism, neoplastic growth and malignancies. The characteristics of the thyroid cell make it a very suitable subject for fundamental radiobiological studies : a sophisticated enzymatic machinery of O_2 metabolism, properties of a differentiated epithelium relevant to carcinogenesis, specialized and easily investigated iodine metabolism. Dosimetry, radiobiology, biochemistry and epidemiology of thyroid irradiation are studied. Newly established model systems may yield end points that are realistically relevant to the two main long term effects, cancer and hypothyroidism, in circumstances where reliable dosimetric information is available and where the modifying effects of hormones and biochemical features can be clarified. The role of intracellular signals on mutagenesis and carcinogenesis will also be investigated.

1. Radiobiology of the thyroid. The radiobiology of the thyroid cell should be studied with accurate dosimetry and using different end points. As experimental model, dog thyroid primary cultures which have retained differentiation characteristics and can be stimulated to grow will be X-irradiated. End points studied are the various steps of iodine metabolism : iodide trapping, its binding to proteins, thyroglobulin synthesis, cyclic nucleotide metabolism and its response to hormones and neurotransmitters, mutation rates and chromosome rearrangements, growth and cell multiplication and effects on the life span of the cells. As a first step, the control of cell differentiation and growth will be further defined and the role of neurotransmitters or metabolites on these processes will be studied and related to the regulation of intracellular signals. These results will be related to the three types of physiological thyroid growth : induced by TSH, reparative growth and developmental growth. The role of TSH in the latter two processes will be tested in rat in vivo using modern techniques of assessing pituitary suppression. The possible role of prostaglandins will be similarly investigated using specific inhibitors. For measurement of mutation rates and chromosomal rearrangement, the techniques developed on V79 chinese hamster cells will be adapted. Resistance to drugs (eg. ouabain) will be the first mutation markers investigated. The life span of thyroid follicular

cells will be investigated in vitro using cloning methods and in vivo under conditions of continuous endogenous stimulation (TRH + goitrogens) to learn whether these cells are terminally differentiated and to provide the necessary background for the investigation of the effect of irradiation on the life span of epithelial cells. The effect of irradiation on embryonal thyroid cells will also be investigated. A number of well defined end points will give a better account of the kinetics and dosimetry of thyroid radiation effects.
The thyroid follicular cell is an interesting model for radiobiology. It is relatively radioresistant with respect to failure to multiply. It forms most of the oxygen derivatives (O_2^-, OH, H_2O_2, etc) which appear in oxygenated irradiated water and presumably contains the enzymatic machinery to detoxify these derivatives. Modulation of the level or activity of these enzymes could therefore allow to study their role in radioresistance. The aim of this work is to define the pathways of oxygen metabolism in the follicular cell and their regulation and to use this model for studying the role of different radicals in radiation effects and, in particular, on the oxygen enhancement ratio for different end points.
2. Irradiation and human thyroid. This project will involve the application to human thyroid of work carried out on experimental models and a cooperative study on risks in human populations previously exposed to radioiodine. The tissue culture methodology will be adapted to human thyroid samples obtained at surgery : the relevant radiobiological end points will be studied in vitro in this material to establish the response of normal human thyroid cells and its alterations in pathological states. This should allow to define the pattern and kinetics of irradiation effects in man and to apply the results obtained on the experimental models (on differentiation, promotion, etc) to prevention or treatment in man. Cell kinetics in human thyroid samples will be studied by in vitro thymidine double labeling to define labeling index, S-Phase, and growth fraction. The G6PDH isozyme pattern will be studied to test the monoclonal or polyclonal nature of irradiation induced nodules and thus the validity of the mutation hypothesis in this case. A cooperative study will be initiated within the framework of the European Thyroid Association. Groups at risk in the population, which have received thyroid doses in the following ranges during the last 20 years (a) 5 - 100 rads b) 100 - 1000 rads and c) 1000 - 5000 rads will be identified. The latter group includes those receiving low dosage radioiodine therapy which has not been proven to be free of carcinogenic risk. An estimate of the number of cases involved will be made before it is evaluated whether and how more detailed studies could be carried out.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-345-81-D

General subject of the contract:

Consequences of radiation exposure : assessment of impairment and restoration of hemopoietic function.

Description of research work:

Investigations should be performed to elucidate the pathogenetic mechanisms that are operative in producing short-term effects of ionizing radiation, to assess the impairment and the regenerative potential of the hemopoietic function and to contribute to the improvement of existing methods to treat the acute radiation syndrome by the separation and cryopreservation of hemopoietic stem cells as the essential cellular elements for the restoration of hemopoietic function. It is the goal of the first project to develop an understanding of the mechanisms underlying the response of hemopoietic cell systems to single, repeated or chronic low level radiation exposure from external sources. In order to achieve this goal, it is essential to improve the existing knowledge on the cellular kinetics of cell renewal systems and their regulatory mechanisms in the target species (man, dog, mouse). The data on the dog and man are fragmentary with respect to bone marrow cell formation and the structure and function of the stem cell system. In parallel with these "physiological studies", it is intended to study in detail the changes that occur in animals (dogs, mice) as a consequence of single, repeated or chronic exposure to radiation. The objective of the studies is to investigate the mechanisms that result in the tolerance of the hemopoietic system to repeated or chronic low level exposure (dose rate < 10 rad/day) and to determine the limits of tolerance since it is known in dogs that bone marrow failure in the form of aplastic anemia or leukemia may develop as a late consequence of such low level long term exposure. Dogs and mice will be used for radiation exposure and their stem cell systems in marrow, blood and extramedullary tissues evaluated as a function of time after exposure. The cell cycle parameters, maturation and turnover times of blood cell function will be studied as well as the content of known humoral regulatory factors. The data obtained will serve to construct simulation models that will reflect the biological data and allow one to predict the early and late response pattern of hemopoietic cell renevall after radiation exposure. The objective of the second project is to study the quantitative and qualitative changes of the blood stem cell system after external and internal single, repeated and chronic radiation exposure. This by assuming that the hemopoietic stem and/or progenitor cell circulating in

the peripheral blood are in a dynamic equilibrium with extravascular pools in bone marrow and spleen and that any whole and partial body exposure will result in an alteration of stem cell system which thus can be detected by blood stem cell changes. Since the late consequences of acute and chronic radiation exposure may well be aplastic anemia or leukemia, it is hoped that the study or blood stem cell changes will allow one to monitor the situation of the hemopoietic stem cell system and to detect early signs of a potential failure at a time when the changes are still reversible. In mice, it is possible to analyse blood derived CFU-S, CFU-C, FBU-E and DFU-Meg.. In dogs, the major test system is the CFU-C assay, while other stem cell assay methods are being developed. In man, the CFU-C and the BFU-E assay systems are available. In order to develop the blood stem cell assay methods into practical devices it will be important to investigate means and ways of automating the procedures and to shorten the culture times. The third project will contribute to the improvement of existing and the development of new approaches for treating the acute radiation syndrome. For clinical use it is important to find non-toxic substances that can increase the number of blood stem cells by mobilization from extravascular storage sites. Such a stem cell mobilization is the prerequistite for the collection of large numbers of stem cells by means of leukocytapheresis. It is then important to improve and standardize the separation of hemopoietic stem and/or progenitor cells from immuno-competent cells by gradient technics.
Furthermore, the knowledge of the cryopreservation of hemopoietic stem and progenitor cells from dogs and man should be improved particularly with respect to the preservation of large fluid volumes containing cells. Finally, it remains to be seen in dogs, in what way one can prevent the development of graft-versus-host disease by the use of purified hemopoietic stem cells in histoincompatible situations. It will be important to establish the principle that the transfusion of immunologically inert stem cell suspensions result in long term chimeras without evidence of gvh. The knowledge gained in animal experiments will be transferred to the clinical level first by using blood stem cells in chemotherapeutically treated patients under autologous conditions and eventually using blood stem cells in patients with marrow aplasia or leukemia in the allogeneic situation. Of great importance will be the search for yet another source of hemopoietic stem cells. It is therefore intended to study in the dog the possiblities of using fetal liver stem cells for the establishement of a "fetal stem cell bank". Studies will include the gestational age of liver, the cryopreservation procedures and the assay methods that best correlate with the short and long term hemopoietic restoration.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-C-355-81-I

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General subject of the contract:
Consequences of radiation exposure and treatment of pathological effects.

Description of research work:
It is well known that one of the major consequences of excessive exposure to radiation or radiomimetic agents is damage to the lymphoid tissue entailing immunodysfunction.
In view of the pivotal role of the lymphoid system in the maintenance of bodily integrity, an in depth elucidation of the mode of action of radiomimetic immunodepressants appears of primary medical interest also considering that immunodepressants are an essential component of the pharmacological treatment in bone-marrow transplantation, an approach of evident interest in the therapy of excessive radiation exposure. A further and more specific background for the studies proposed can be recognized in a series of previous recent studies of this laboratory evidentiating that cytotoxic immunodepressants even when chemically closely related and generally credited with equal molecular modes of action, are in reality substantially heterogenous in their effect on the various cell types constituting the immune system. This heterogeneity is not only quantitative (i.e. in the degree of inhibition exerted on immunocyte population or subset) but also qualitative (i.e. certain immunocytes are sensitive to a given drug but resistant to other chemicals even of the same class) with an extra complexity represented by the fact that, at least in given conditions of treatment, instead of inhibition of immunity, enhancement of immune function can be observed. Such heterogeneity can be important at both basic and applied levels. In fact these studies can one hand be of interest in gaining a better knowledge of the biology of the immune system. In addition, a better understanding of the heterogeneity of immunodepressive agents can be in principle of therapeutic relevance; for instance, a differential interaction of chemicals with Natural Killer (NK) cells, believed to be involved in the rejection of Hh incompatible tissues in addition to the resistance to infectious agents, may be of importance in a safer therapy of bone marrow transplantation.
On this background and as detailed below, the research program proposed involves the extension of previous studies to a novel series of immunodepressive agents (e.g. nitrosoureas such as BCNU, MethylCCNU OH-ethylCNU and Chlorozoticin, cis-Platinum, Vincristine and Vinblastine, 5FU, Methotrexate and other antifolates), which will be investigated initially for their effect macrophages and NK cells (i.e. the elements sustaining natural resistance mechanisms) taking also into

account the possibility that those cells when taken from different districts may also exhibit differential susceptibility to the modulatory activity of these compounds. Another immunocyte population to be investigated will be represented by so-called regulatory or suppressor cells. Whereas a limited number of drugs have been investigated for their effects on T regulatory cells, there is still need to investigate a large number of compounds and to investigate cytotoxics for their modulatory activity on macrophagic suppressor. Another objective of these studies will be the investigation of the capacity of immunodepressants radiomimetics on the production of selected representative monokines-lymphokines and on the sensitivity of the appropriate target cells to these immunological mediators.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-347-81-F

General subject of the contract:

Experimental study of acute localized radiation lesions in pigs.

Description of research work:

The increasing use of nuclear techniques in industry, medicine and research as well as accidents due to heterogeneous, local, external radiation warrant an experimental study in depth on the evaluation of the absorbed doses, the pathogenesis of the radiation lesions and their evolution. The aim is to gather as quickly as possible enough information for a prognosis and if appropriate, for the development of therapeutic methods.

The choice of experimental species is fundamental to an extrapolation to man. The pig is being proposed on the following grounds : stature, weight, physiological corpulence comparable to man, unpigmented skin and absence of fur. The stature, skin, muscular tissue volume, deep-seated bone diseases, neurovascular bundles, etc., pertaining to the animal afford a wide variety as regards the choice and extent of the territories to be irradiated. These conditions therefore permit full-scale simulation of a radiological accident. It is known, moreover, that pigs have already been widely used in radiobiological studies of this kind. Their life-span is also a valid argument in that it facilitates medium and long-term observations.

As the means for carrying out the irradiations, we propose the use of an industrial gamma-radiography apparatus with an iridium-192 source of about 40 Ci. The dose range to be explored would be about 3.000-15.000 rad at the surface.

The studies to be carried out on the animals irradiated locally in various parts of the body (limbs, thorax, abdomen) are broken down into :

- physical dosimetry studies of iridium-192 radiation; determination of the absorbed doses at the surface and at depth in the various organs and tissues;
- clinical studies on the development of the radiation lesions, and their reparation and long-term evolution as observed over two years;
- biological dosimetry studies - cytogenetics of fibroblast culture;
- histopathological studies of the irradiated organs and tissues;
- early functional studies at the surface (thermography and effusimetry) and later functional study at depth (scintigraphy, radiography, arteriography, measurement of arterial pressure).

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-348-81-F

General subject of the contract:

Physiopathological study of localized radiation injury : diagnostic and therapeutic implications.

Description of research work:

Despite significant advances, therapy of localized radiation injury is still often symptomatic and results in radical resection or amputation surgery. The recent successes in repair surgery (flap technique, microsurgery) depend to a large extent on information supplied to the surgeons by radiopathologists.

Thus the trophic situation of the irradiated tissues and their interface with the healthy tissues should be known for conservative medical treatment and for the decision to intervene by surgery. This can only be achieved by means of a multi-disciplinary strategy which lends to paraclinical examinations. Adequate means must be made available if it is wished to obtain results : the situation is urgent since an increase in accidents is currently being observed.

The research work is conducted in such a way as to indicate :

- early diagnosis,
- the clinically insignificant manifestations of the latent periods,
- the risks of secondary necrosis,
- a clinical estimation of the dose,
- mechanisms which make therapy possible,
- the effectiveness of the therapies used,
- the trophic situation of the tissues when surgical decisions are to be taken,
- the stability or pattern of evolution of the after-effects.

The tissues to be explored first are : the skin, the connective tissue, the vascular system, the bones, the muscles, the nerves and the bone marrow.

A. Diagnostic methods

1. Thermal methods
   These methods include, in particular : effusion capacity or thermal extraction coefficient of the tissues, the atraumatic determination of which explores depths of several millimetres and provides information on the microcirculation, different methods of thermography (plate and camera), evaporimetry.
2. Electric methods
   Measurement of the impedance of the tissues, particularly of the skin, and plethysmography by impedance measurement are two indispensable methods.

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3. Optical methods
The following methods will be developed : different forms of photography, colorimetry, optical plethysmography, and capillaroscopy
4. Nuclear methods
Measurements of cutaneous isotope elimination, circulatory flow rates, in the fixation bones.
5. Biochemical methods
Determination of local or systemic markers of inflammation and damage to tissues and cells (cutaneous pH and pCl, coagulation and inflammation proteins, proteases and antiproteases, histolysis markers, etc...) proteolytic activity of irradiated tissues.
6. Biological methods
These methods will mainly concern bone-marrow and stromal cell culture and cytogenetics aiming at quantitative diagnosis.
7. Miscellaneous methods
Of the classical paraclinical examinations, circulatory rates will be measured by means of the Doppler effect
are of particular interest.

B. Therapeutic implications

All these examinations should contribute to establishing surgical decisions. The problem of grafts and cutaneous flaps will be studied together with the provisional covering of tissues with foreign material (pigsking). Dermato-autoplasty by culturing basal cells of the epidermis will be developed.

The activity of therapeutic agents such as heparine, vasodilators and the cicatring agents will be checked by means of these methods together with the effectiveness of physiotherapeutic methods : heat, ultrasonic, HF.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-346-81-F

General subject of the contract:

Study of the influence of the rate and the fractionation of the dose on the biological effects of gamma radiation at medium and high doses.

Description of research work:

This programme consists of two projects, namely, haematology and anatomopathology on the one hand and chromosomal anomalies on the other hand.

1. Haematological and anatomopathological studies
   A. Haematological studies
      Two rates have been adopted; i.e., 10 rad/h and 1 000 rad/h. At 10 rad/h, the number of weeks and number of hours per day will be varied so as to have overlapping doses. The series of doses chosen are : 600 rad (50 rats), 1 100 rad (50 rats), 1 700 rad (50 rats), 3 000 rad (50 rats) and 4 000 rad (20 rats). The irradiation of these animals will be carried out as early as the first year of the contract, whilst the study of the various cell populations of their blood will be carried out at different intervals of time, the measurements being taken during the irradiation process. Long-term changes will also be measured. At 1 000 rad/h, the experimental pattern adopted is : 600 rad (40 rats), 400 rad (40 rats) and 200 rad (40 rats).
      The haematological studies will be carried out as specified in the preceding paragraph. These irradiations will be carried out in the second year.
   B. Anatomopathological studies The long-term effects of radiation on the organs of the 340 rats irradiated for the purposes of the haematology report will be observed and there will be a waiting period for the detection of tumours, in particular those of a malignant character. These results will be compared with those already obtained from rats of the same strain which have been irradiated with neutrons and in which more than 500 cancers of the various organs have already been detected. As the average survival time of the rats exceeds two years, these studies will be mainly carried out during the last two years of the project.
2. Chromosomal aberrations. The first objective of this study is to investigate an animal species whose kinetics of the lymphocytic anomalies approximate to those of man and then to study, in that species, the role of the rate and fractionation of the dose.

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The first stage could be completed in 18 months. This investigations will be carried out on two species, namely, the rabbit and a primate.

Initially, the doses of acute irradiation giving rise to thromobocytopenia without lymphocytopenia will be ascertained for each species. A study will then be made of the changes in the chromosomal anomalies in samples taken on the day of the irradiation compared with those taken 15, 30, 60, 90 and 120 days afterwards. Nearly 5 000 cells in all will be analysed. The objective of the second stage will be to study, on the chosen species and using the same system, the role of the rate and fractionation of the dose. This study is planned to take two and a half years and more than 10 000 cells will be analysed.

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Head(s) of research teams(s):

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Contract no.: BIO-C-352-81-F

General subject of the contract:

Influence of ionizing radiations on the in vitro aging and immortalization of fibroblasts.

Description of research work:

The long-term objective of the project is to identify the injuries responsible for increasing sensitivity to low doses of radiation during the aging of fibroblasts and to determine whether these injuries are also responsible for cellular aging. In addition, it will be attempted to determine whether the fibroblasts of certain high cancer-risk patients respond to radiation in a particular way.

The approach at molecular level will concern the detection of sites sensitive to the S_1 and gamma endonucleases and to alkaline pH. These sites have been described during aging in vivo and, during phase III of embryonic fibroblast, an increase of the alkali-sensitive sites of the DNA was discovered after alkaline sucrose gradient centrifugation. In order to determine whether there is a relationship between the appearance of these sites and sensitivity to radiation, a comparison will be made between cells in which the division potential is reduced, cells in which the potential is prolonged and cells in which it remains unchanged. This will be done by alkaline sucrose gradient centrifugation before and after treatment with the S_1 and gamma endonucleases.

An attempt will also be made to determine whether the results obtained with the fibroblasts from patients suffering from the Fanconi syndrome are also obtained with fibroblasts from donors suffering from other genetic disorders likely to lead to cancer. If there is no relationship between this phenomenon and the risk of cancer it will then be used as a tool for understanding the associated genetic modifications.

1. Effect of low doses of ionizing radiation on the life expectancy of fibroblasts from normal and high cancer-risk human donors.

The cell cultures will be irradiated with a Co-60 source, 12 hours after the sub-culture, with a dose of 100 rad at a rate of 0.27 rad/min. The irradiation will be repeated every two runs up to 500 rad. Six groups will therefore be obtained : controls and cultures irradiated once, twice, three times, four times and five times. The room in which the irradiation will be carried out is divided in two by a lead wall. On either side of the wall is a plexiglass platform heated by circulating water, assuring the same temperature of both platforms. One of the platforms is placed above the Co-60 source and the other is used for the control cultures.

After irradiation, the cultures will be replaced in the drying cabinet until the rest phase. Then they will be trypsinated, counted with a Coulter counter and sub-cultivated in new flasks at a ratio of 1:2. The survival curves will be expressed as a function of the maximum densities before each sub-culture. The colonies will be maintained until the division potential is exhausted. The total number of cells produced during life in culture will be calculated by adding the difference between the number of cells at saturation density and the number of cells innoculated at the moment of the sub-culture. These experiments will be carried out with fibroblasts from normal donors and donors of the same age suffering from Down's syndrome, tyrosinosis, xeroderma pigmentosum, Cockayne's syndrome and Fanconi's anaemia. The experiment will be repeated with fibroblasts from donors suffering from the last mentioned disorder in order to determine whether the same results are obtained with different forms of the same disorder. It is also intended to carry out the same experiments for Fanconi's anaemia with fibroblasts from donors who are heterozygote. All biopsies will be taken from the upper surface of the forearm. The purpose is to determine whether radiation has the same effect on different genetic high cancer-risk syndromes.

2. Search for modifications in the DNA

This will be performed every five runs during the lifetime of the colonies of fibroblasts of the three types selected in accordance with response to radiation, that is to say, impaired, prolonged or unchanged division potential. An attempt will be made to reveal the presence of single-strand breaks in the DNA, distortions of the DNA chain and alkali-sensitive sites. This will be a first step towards the detection of modifications in the DNA which might cause an increase in the fragility of the molecule. The S_1 endonuclease cuts the DNA single strand and the gamma endonuclease cuts the molecule at the point of the distortions. The alkaline-sucrose grandients will reveal the DNA cut by the enzymes and the presence of alkali-sensitive sites. It is therefore intended to determine whether the injuries play a role in the response to low doses of radiation.

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Head(s) of research teams(s):

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Contract no.: BIO-C-353-81-I

General subject of the contract:

Physiopathology and therapy of radiation induced marrow aplasia : role of glycoprotein hormones modulating early hemopoiesis.

Description of research work:

Normal hemopoiesis is maintained by a pool of pluripotent stem cells (CFU-S*) : these are capable of both self-renewal and differentiation into hemopoietic precursors (BFU-E, CFU-E, GM-CFU-C, etc*), which in turn feed the respective differentiated lineages (erythroid, myelomacrophage, megakaryocyte, lymphoid). Exposure to radiation (or injection of radiomimetic agents) induces a dose/related depletion of hemopoietic stem cells and precursors, which may lead to marrow aplasia. Post-radiation recovery of hemopoiesis is dependent upon restoration of a near-normal number of stem cells and precursors. A key aspect of radiation-induced aplasia and post-radiation recovery is thus represented by mechanisms regulating the kinetics of stem cells and precursors, both under normal conditions and following radiation.
Recent advances in this field may hopefully lead to unveil these mechanisms in the near future. In particular, a new class of glycoproteins (BEF, Ep, GM-GSF, M-CSF*) modulating cycling and differentiation of stem cells and precursors has been identified and partially purified. It is proposed that studies on these mechanisms, and more particularly on the above glycoproteins, may allow a dramatic advance in our understanding of radiation-induces aplasia and post-radiation recovery, thus perhaps leading to establish new strategies and technical tools for prevention and treatment of these conditions. 1. Background a) Hormone purification. Ep, GM-CSF and M-CSF have been recently purified. Purification of BEF and other modulators of early hemopoiesis is still unsatisfactory (vide infra). b) Role of BEF. Ep largely controls the kinetics and differentiation of CFU-E, as well as the erythropoietic rate. In vitro, BEF allows survival of BFU-E, enhances BFU-E cycling and promotes burst formation. In vivo, fluctuations of BEF levels may modulate the kinetics of BFU-E and CFU-E pools, to respectively a major and minor extent. c) Relationship between BEF, GM-CSF and other modulators of early hematopoiesis. BEF without Ep promotes GM-colony growth; purified GM-CSF, in the presence of Ep, exerts as BEF-like action. A crucial question concerns, therefore, the possible identity of BEF and GM-CSF, and more generally the relationship between recently-described factors acting on early hemopoietic precursors. In this regard, Wagemaker apparently separated three slightly distinct glycoprotein factors : (a) the first one enhances proliferation and differentiation of primitive BFU-E (BEF sensu strictu); (b) the second one promotes

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proliferation and differentiation of a primitive myeloid progenitor (giving rise to progeny responding to GM-CSF); and (c) the third one induces proliferation of CFU-S. 2. Specific aims. It is proposed to investigate the following aspects: a) fluctuations of the serum activity of modulators of early hemopoiesis (BEF and then other factors) following radiation or radiomimetic agents. Preliminary evidence indicates that BEF levels are enhanced in these conditions : it is proposed to examine in detail fluctuations of BEF levels after acute of prolonged radiation, as well as treatment with radiomimetic agents (this study is also essential in view of the following aims). Fluctuations of other modulators of early hemopoiesis (GM-CSF, "CFU-S factor", etc) shall be assayed too, in case of these factors prove to be different from BEF. b) Purification and biochemical characterization of NEF produced in irradiated mice. Purification of BEF from medium conditioned by lectin-stimulated splenic cells of normal or irradiated mice via a combination of A) DEAE-cellulose chromatography, B) Sepharose-Concanavalin A affinity chromatography, C) Sephadex G-150 gel filtration, and D) polyacrylamide gel electrophoresis. Purified murine BEF shall be compared with purified GM-or M-CSF, as well as with similarly purified BEF from irradiated mice, in respect to its biological activity (BFU-E and GM-CFU-C growth and proliferative rate; CFU-S cycling) and physicochemical properties (in particular, comparison of elution prolfiles, also possibly employing I-131 labelled, purified GM- or M-CSF). This study seems of key relevance, in that it aims to clarify whether or not GM-CSF is identical with 1) BEF from normal and irradiated mice, 2) factors postulated to act on CFU-S or early GM-CFU-C. c) Role of the T-lymphocyte-macrophage system in radiation aplasia. Preliminary results from our laboratory indicate that injection of crude BEF in mildly-irradiated mice enhances their hemopoietic recovery. In this regard, the T-lymphocyte-macrophage complex plays a major role in production of BEF, GM-CSF, and possibly of the other, related glycoproteins. Although the macrophage is somehow resistant to radiation, T-lymphocytes (and particularly the "helper" subpopulation) are fairly radio-sensitive. Thus, damage of T-lymphocytes, and possibly macrophages, may dampen the BEF (+ GM-CSF, etc) response to at least some types of radiation. Studies are therefore proposed to monitor a) BEF (+ GM-CSF, etc) activity, b) kinetics of stem cell and precursors in mice subjected to I) syngeneic T-lymphocyte transplantion + irradiation, II) macrophage hyperplasia (via Zymosan injection, etc) + irradiation, as compared to controls subjected only to irradiation.

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* Abbreviations : CFU-S, CFU-E, GM-CFU-C : colony-forming unit, spleen, erythroid, granulo-monocytic. BFU-E : burst-forming unit, erythroid. BEF : burst-enhancing factor(s). Ep : erythropoietin. GM-CSF, M-CSF : colony-stimulating factor, granulo-monocytic, monocytic. FCS : fetal calf serum.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-C-290-81-D

General subject of the contract:

Investigation on biological dosimetry : Formation of micronuclei in mammalian cells after irradiation with neutrons.

Description of research work:

It has repeatedly been observed that after cell division, which takes place after a radiation exposure, smaller chromatin pieces appear outside the cell nucleus. These chromatin pieces are called micronuclei. Cytogenetic investigations have shown that the micronuclei can be caused by radiation induced chromosome breaks and loss after mitotic cell division. At a meeting, which has been organized in December 1979 at München-Neuherberg by the World Health Organization, it has been recommended to use the quantitative determination of micronuclei as a cytogenetic parameter for "biological dosimetry". In comparison to chromosome analysis the micronucleus test has the advantage that it is much faster and can be performed with less expenditure.

While a number of such studies has been performed after exposure with low LET radiation similar data have not been reported for neutron irradiation. Therefore the formation of micronuclei will be investigated after neutron irradiation (cyclotron). The neutron irradiation will be undertaken by the "Abteilung für Medizinische Strahlenphysik" of the "Institut für Medizinische Strahlenphysik und Strahlenbiologie". The following biological systems will be used for the experimental studies : 1) Preimplanted mouse embryos, 2) human tumor cells, cultured in vitro, 3) human lymphocytes after PHA-stimulation.

The preimplanted embryos can be cultured in vitro under nearly physiological conditions. These early stages of mammalian embryogenesis are extremely radiosensitive as shown after X-irradiation. Dose effect relations will be determined after exposure with neutrons and other radiation qualities (X-rays, Cobalt-60 gamma-rays, photons). As this biological system responds to radiation doses of a few Rads and as a wide dose range can be studied these studies can be used as a "radiation quality dependent biological dosimeter".

The formation of micronuclei is dependent on cell proliferation. In order to use the test as a "biological dosimeter" it is absolutely necessary to include these parameters and studies on the mechanism in these investigations. Under consideration of such data RBE-values will be determined in the low dose range.

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Also these data will give informations on the alterations of early embryogenesis after neutron irradiation.
Besides the determinations with the very radiosensitive embryos it appears useful to perform such studies with more resistent, proliferating mammalian cells to give the test a broader base. As neutrons inhibit recovery processes the dose effect relationship will be studied with cells which exhibit these processes in a considerable way. Such cells are for instance melanoma cells. These cells will also be cultured in vitro, in order to include proliferation kinetics. For the evaluation as a "biological dosimeter" experiences are necessary with a broad spectrum of cells.
The investigations will be further extended to human lymphocytes. At first lymphocytes will be irradiated with neutrons and other radiation qualities in vitro. For the determination of micronuclei it is necessary, to stimulate the proliferation of lymphocytes with a mitogen, like for the chromosome analysis. Also with this system dose effect relationship and RBE-values will be determined. It will then be possible to obtain lymphocytes from patients, who have been irradiated for tumor therapy with neutrons, and to count the micronuclei after PHA-stimulation. On the basis of the data from physical dosimetry it will be possible to obtain further information for the usefulness of the test system as a "biological dosimeter" after irradiation with neutrons in vivo.

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Head(s) of research teams(s):

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Contract no.: BIO-C-364-81-EIR

General subject of the contract:

Thyroid irradiation : dosimetry and radiobiological consequences in cell culture systems and in humans.

Description of research work:

Consequences of deliberate and accidental thyroid irradiation are well documented and include induced malignancies, other neoplastic abnormalities, and hypothyroidism. Biochemical, hormonal and dosimetric factors are particularly important in the induction and development of these effects. Despite the extent of thyroid induced radiation effects very little is know about many aspects of their development and radiobiology, probably because of difficulties in dosimetry, the absence of biological models that rendered precise studies possible, and the complexity of thyroid biochemistry.

In collaboration with Dr. J.E. Dumont it will be attempted to correct some of these deficiencies in knowledge by undertaking, with new methodologies, studies involving the (a) dosimetry, (b) radiobiology, (c) epidemiology and (d) biochemistry of thyroid irradiation. In particular, it is intended to derive from newly established model systems end points that are realistically relevant to the 2 main long-term effects of thyroid irradiation (cancer and hypothyroidism), in circumstances where reliable dosimetric information is available and where the modifying effects of hormones and special biochemical features can be clarified.

The first project is concerned with the dosimetry of radionuclides of iodine in the human thyroid and in tissue culture systems. With regard to the human thyroid the project is intendedto further simplify the methods involved in obtaining information on iodine kinetics for human dosimetry. In essence it is expected to eliminate the need to determine the activity-time curve in detail, and replace it with a single reading obtained from thermoluminescent discs placed over the gland for the entire period required. The discs will integrate the activity present over the period observed and require a further single reading, possibly an external count to allow the integral be expressed in terms of the absolute activity in the gland. The programme will be concerned with the development, validation and implementation of this method in practice. A reliable and relatively routine method of gland mass estimation based on ultrasound techniques, and comparison of the results with activity distributions obtained from scintiscanning will be sought. The ultrasonar methods require considerable methodological development over and above conventional thyroid ultrasound scanning. Finally, the study will be concerned with the effects of radioprotective measures on the kinetics of injected radionuclides and

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on their microdosimetry in glands that are surgically excised shortly after radionuclides have been administered. To allow radiobiological investigations be performed in differentiated thyroid cell cultures detailed quantitative studies of the geometry of the cultures and the distribution of radionuclides through them will have to be made. This will involve a study of the number, size, shape and distribution of follicles present in the cultures as well as the extent to which activity is concentrated in them.

The second project will examine the radiobiology of the thyroid using cultured sheep thyroid cells with respect to the effects of single doses of X-rays on cell survival, iodide trapping, the extent to which differentiation is preserved, hormone indices of cell function, and induction and expression of chromosome abberations. In studying these effects the influence of the time of irradiation and the kinetics of the appearance of the effects will be optimised. It is intended to develop a number of new endpoints for the model involving transformation assays, and indices of dedifferentiation/ redifferentiation at a refined level using the detailed knowledge of thyroid function available. The above studies will include split-dose X-ray investigations, and eventually will include determination of the effects of I-131 and I-125 irradiation using dosimetric results from Project 1. It is intended to quantitatively correlate the results from single and split X-ray doses with those from radionuclide irradiation and thereby account for the dose-rate effects involved with the latter. Finally, the effect of thyroid metabolism on its radiobiological response will be determined.

In the third project, the tissue culture system will be extended to study various types of human material, and aid with identifying human groups at risk from thyroid irradiation. The method will be applied to surgically excised normal and pathological human thyroid tissue. Hopefully, the development will allow small amounts of tissue, such as that obtained at biopsy, be successfully cultured. The relevant radiobiological endpoints will be studied to determine how reproducible/variable the response of the human gland is in healthy and diseased states. The methodology should offer an opportunity to study endpoints such as chromosome abberations in glands excised at various times after radioiodine scintiscans or uptake tests where relatively good dosimetric information is available. It should also allow the extent to which hormonal and biochemical factors as well as partial dedifferentiation influence the response, be studied in the light of the results in Project 2. Groups at risk will be identified in populations who have received thyroid doses in the following 3 ranges during the last 20 years (a) 5-100 rads ; (b) 100-1000 rads and (c) 1000-5000 rads. The latter group includes those receiving low dosage radioiodine therapy which has not been proven to be free of carcinogenic risk. Following estimates of the numbers in each category, an evaluation of whether more detailed studies would be desirable will be made. New formulations of benefit risk analysis will be developed with respect to two forms of radiation induced non fatal harm in the gland, hypothyroidism and benign neoplasia.

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Head(s) of research teams(s):

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Contract no.: BIO-C-344-81-D

General subject of the contract:

Treatment of short term effects of radiation injury to the lymphohemopoietic system.

Description of research work:

In spite of recent progress, radiation-induced bone marrow damage cannot be treated satisfactorily without further analysis of immunological and hematological factors affecting cellular repopulation of irradiated bone marrow. The proposed research program therefore concentrates on the following experimental as well as clinical projects:

1. Recovery from radiation injury after maximal doses of fractionated total and partial body irradiation and bone marrow transplantation. These studies will be undertaken in dogs, an animal model which in contrast to rodents permits the collection, preservation and transfusion of allogeneic as well as autologous bone marrow into individual animals. The objective is to raise radiation tolerance by fractionating the total radiation dose, by varying the dose rate and by treating radiation induced bone marrow failure with bone marrow transplantation. The determination of maximal radiation injury to the hemopoietic system in the absence of irreversible damage to other vital tissues such as the gut will also help define a regimen applicable to a more complete eradication of leukemic and other tumor cells. Fractionated irradiation and provoked mortality after 2-3 times 600 R preceeding 1200 R will be followed by allogeneic DLA, MLC compatible or incompatible bone marrow transplantation of autologous bone marrow preserved in liquid nitrogen. Various dose rates (e.g. 11,22, 50 R/min) applied under the conditions of described before will be applied.

2. Removal by antisera of anti-host immune reactivity in irradiated bone marrow recipients. Elimination by antisera of cells responsible for graft-versus-host (g-v-h) reactions will be tested for their suppressive effect on g-v-h. Previous studies with absorbed anti-T cell globulin in experimental animals will be extended to the suppression of g-v-h in leukemic patients treated with total body irradiation and bone marrow transplantation. In rodents antisera against early or late T cells will be investigated for their g-v-h suppressive potential. Suppression of g-v-h after transplantation of bone marrow pretreated with anti-T cell globulin will be studied in leukemic patients conditioned with total body irradiation. Suppression of g-v-h will be studied in rodents following incubation of donor marrow with anti-early or -late T cell globulin.

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3. Repopulation of the lymphatic tissue after total body irradiation. An immunohistochemical method has been developed for the demonstration of surface markers in tissue sections. This permits to study the distribution of T and B cells in lymphatic tissues recovering from total body irradiation and bone marrow transplantation. Histology of lymphatic tissues various days after transplantation of H-2 incompatible marrow pretreated with anti-T cell globulin will be done as well as immunohistochemical staining of T and B cell areas in unthymectomized or thymectomized chimaeras.

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Head(s) of research teams(s):

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Contract no.: BIO-C-357-81-NL

General subject of the contract:

Development of safe procedures for transplantation of mismatched bone marrow.

Description of research work:

The bone marrow transplantation program in Rijswijk comprises research in rodents directed at the fundamental aspects of bone marrow transplantation, and preclinical research with rhesus monkeys and dogs which is the subject of the present study. In the latter species, outbred like man, the major research objective is the development of a method that will make bone marrow transplantation a realistic treatment protocol for each radiation victim that can benefit from these procedures. Simplification of bone marrow transplantation and improvement of its safety is the second research aim. These efforts will be undertaken to promote a more general application of bone marrow transplantation.

In the past few years, major progress has been made in identifying those factors which beneficially modify the double immunological barrier of bone marrow transplantation. These are rejection of the bone marrow transplant host-versus-graft (HvG) and, if successful engraftment is accomplished, graft-versus-host disease (GvHD). Most of the factors which determine take and GvHD have now been identified (see table) :

Major factors determining :

Acceptance of allogeneic bone marrow graft	type and severity of GvHD
1. degree of immunogenetic compatibility between donor and recipient.	1. idem
2. Composition of the hemopoietic graft : ratio of hemopoietic stem cells and lymphocytes.	2. idem
3. the number of cells transplanted.	3. idem
4. preparation ("conditioning") of the recipient.	4. sex of donor
5. prior sensitization of the host, e.g; by transfusions.	5. composition of gastro-intestinal microflora of the recipient.

The present study envisages a more detailed analysis of these factors so as to enable their practical application in humans.

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Under project 1 studies will be done to provide effective conditioning regimens which allow the rapid take of mismatched lymphocyte-depleted stem cell concentrates and which further define the influence of the various MHC loci on the take and the incidence of GvHD.
Under project 2 the mechanism of the beneficial effect of bacteriological decontamination on GvHD will be further elucidated so as to define the most effective and practical methodology for clinical application.
Under project 3 the immunological reconstitution of mismatched radiation chimeras will be studied because such information is needed before the use of mismatched bone marrow grafts can be put into clinical practice. Such information is also required as a baseline for eventual attempts at stimulating immune recovery.

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Head(s) of research teams(s):

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Contract no.: BIO-C-471-81-US

General subject of the contract:

Clinical investigations on bone marrow transplantations

Description of research work:

The International Bone Marrow Transplant Registry (IBMTR) is a working group of clinical investigators with participation by almost all bone marrow transplant teams in the world. The teams report comprehensive information regarding their consecutive bone marrow transplant experience to the IBMTR. The data are placed on the computer of the Statistical Center, located at the Medical College of Wisconsin. Pooled data are analyzed at frequent intervals to determine those factors which influence success and failure of marrow transplantation. The results of the analyses are presented at international scientific meetings and published in high circulation medical journals and textbooks. All work is under the immediate direction of Dr. Mortimer M. Bortin and Dr. Alfred A. Rimm and is supervised and managed by an Advisory Committee whose members are renowned bone marrow transplanters with a wide range of specialized disciplines within the field. The Advisory Committee is responsible for the design of clinically relevant analyses and for the scientific validity of IBMTR reports.

The overall purpose of the International Bone Marrow Transplant Registry (IBMTR) is to aid in improving the success rate of bone marrow transplantation as applied for the treatment of patients with a variety of otherwise incurable diseases.

The specific aims of the IBMTR are :

A. To maintain a statistical center for the collection, organization and analysis of clinical data provided by bone marrow transplant teams throughout the world ;

B. To disseminate the results of clinically relevant analyses of pooled IBMTR data to bone marrow transplant teams, and to the medical profession for the earliest possible benefit to patients who might be helped by bone marrow transplantation treatment ; and

C. To aid in designing, organizing and providing statistical support for controlled, cooperative clinical trials utilizing bone marrow transplantation.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-376-81-NL

General subject of the contract:

Fundamental studies on the possible mechanisms of radiation carcinogenesis.

Description of research work:

For the estimation of the carcinogenic risks of low doses of radiation, it is of importance to gain more insight into the exact mechanisms of radiation induced neoplastic conversion of cells, as direct estimates of the cancer incidence cannot be obtained from epidemiological nor from experimental studies for the dose range below approximately 10 rad. The hypothesis has been developed in our institute that radiation carcinogenesis may involve the release of DNA fragments from lethally damaged cells which may become integrated with the genome of other damaged cells during their repair process. Such an event might result in an uninhibited expression of genes involved in cell proliferation or in the blocking of genes for differentiation when the appropriate DNA fragments are incorporated in sites of the genome, leading to neoplastic conversion. Support for this hypothesis came from results of experiments with in vitro cultured cells who showed that the incidence of malignant transformation induced by irradiating cultured fibroblasts could be matched by inflicting the same amount of damage in the population by means of mechanical cell kill or cell kill by repeated freezing and thawing. These results could not be explained by assuming the presence of oncogenic virus or provirus.

More recently it has been found that under certain conditions normal murine 3T3 cells (BALB or NIH) can be transformed in vitro by mechanically fragmented DNA from different organs of germfree BALB/c mice. These conditions are ageing of the cultured cells or preinfection with a non-transforming murine leukemia virus. The transformed cell lines produced tumors in syngeneic mice.

It is intended to establish whether the transforming DNA fragments occur in other mouse strains as well, which is to be expected in view of the previous results. The next step is to define the DNA fragment or fragments which possess the capacity to induce malignant transformation in normal cells. To this end normal cellular DNA will be fragmented with a number of bacterial restriction enzymes and the resulting products will be assayed for transformation. The ultimate purpose of this approach is the isolation and characterization of the transforming sequences in normal cellular mouse DNA by means of molecular cloning. By means of repeated separation by agarose gel electrophoresis the average size of the transforming segment will be

determined. The fragments of a certain size class will be propagated in Escherichia coli with a "charon" vector by means of the recombinant DNA technique. The various cloned molecules will be tested for the transforming capacity on 3T3 cells.
By means of molecular hybridization with representative c-DNA probes and immunoprecipitation with antisera specific for the onc-gen product of known oncoviruses, it will be investigated whether the cells transformed by normal cellular DNA have an increased number of the sequences and uninhibited expression of genes related to acute-oncogenic murine viruses. It is known that this kind of viruses results from the recombination of leukemia viruses and normal cellular sequences.
Subsequently, a number of radiation-induced mouse tumors (in particular sarcomas and leukemias) will be investigated by means of the so-called Southern blotting technique for possible amplification of the transforming sequences, which have been purified by molecular cloning. When an antiserum is available to the protein coded for by this DNA fragment, such tumors will be studied by means of the immunoprecipitation technique for uninhibited expression of these genes.
The conditions under which normal cells can be transformed by fragments of normal cellular DNA will also be analyzed further. In particular, it will be investigated whether low doses of radiation (below 20 rad) will make 3T3 cells as well as secondary mouse embryo fibroblast cultures permissive to neoplastic conversion by normal cellular DNA fragments.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-375-81-NL

General subject of the contract:

Influence of repeated low dose irradiation on mammary gland carcinogenesis.

Description of research work:

There are strong indications that mass population screening, employing clinical examination and mammography, results in cancer detection at an earlier stage, with consequent improvement in cancer cure and survival rates. An important condition, however, is that the dose level per examination should be reduced to a maximum of 2 rad in order to make mass mammography acceptable from the point of view of the added risk of breast cancer induction.

In earlier mammographic procedures the entrance doses were in the order of 10 to 15 rad, whereas exit doses were reported to be in the order of 200 mrad. Recent techniques, such as the use of intensifying screens in combination with new film materials, however, made it possible to decrease the entrance dose in the breast by a factor of 30, resulting in a dose value of 0.4 to 0.5 rad without unacceptable loss of diagnostic information. In general, a total number of three fields is employed in a single mammography session, which could bring the total entrance dose up to 1.2-1.5 rad. This maximum dose value of 1.5 rad per investigation can be taken as representative for the human situation in the near future. The dose absorbed in the cells at risk for mammary cancer induction will be somewhat smaller depending on the geometry of the female breast. Since the X-ray doses absorbed in relevant target volumes of the breast during mammography are around 1 rad and because the number of diagnostic evaluations during a lifetime is expected to be about 10, the total dose in relevant target volumes will be approximately 10 rad.

Risk estimates for tumour induction can be based on studies executed in groups of animals e.g. several strains of rats. Basic information has been obtained about the tumour incidence in different rat strains after single dose irradiations with X-rays and monoenergetic fast neutrons. The probability of animals surviving without known mammary tumours as a function of time, has been calculated according to a life table analysis. Different subgroups, notably intact females, intact females with estradiol 17-beta and hystero-ovariectomized females, were included in the study.

For an evaluation of the risks of repeated low dose irradiation spread over periods of weeks or months, a situation generally encountered in

radiation protection, the results of these single-dose irradiations will be used as a base line. In order to extrapolate data on tumour induction obtained with large doses to the region of much smaller doses which are pertinent to the present study, the shapes of dose-incidence relationships and the influence of a number of factors will be evaluated.

The relative contributions from repair of sublèthal damage, repopulation and tissue turnover during the course of a fractionated treatment will be investigated. It is expected that the influence of some of these factors will diminish considerably if radiation with a higher LET is employed. Consequently, parallel experiments with high LET radiations preferably with neutrons of 0.5 MeV energy will be executed.

Main emphasis will be placed on the influence of repeated low dose irradiation with X-rays (10 fractions of 4 rad compared with a single dose of 40 rad) and 0.5 MeV neutrons (10 fractions of 1 rad compared with a single dose of 10 rad) on mammary gland carcinogenesis in two rat strains. The histological examinations will allow a scoring of benign versus malignant lesions, the latter being only of relevance for the evaluation of risks in man.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-372-81-F

General subject of the contract:

Study of uranium metabolism and of actual health hazards in the uranium industry (natural and enriched).

Description of research work:

The uranium industry in France makes use at different stages of numerous types of compounds, oxides such as UO_3, UO_2, U_3O_8, uranates, fluorides such as UF_4, UO_2F_2, UF_6, etc.) for varying percentages of U-235 enrichment (natural, depleted, enriched).

The exact toxicity and metabolism of these different forms are, on one hand, not sufficiently known, which renders hazardous the interpretation of the results of radiotoxicological tests carried out on the workers; on the other hand, the observations published in the literature seem contradictory. It is proposed to extend the current research programme in order to determine accurately the actual toxicity and the metabolism of the uranium compounds used in the French nuclear industry.

1. Study of the pollutant and the conditions of exposure.

In order to interpret radiotoxicological results correctly, the parameters and conditions of occupational exposure must be known. A prerequisite for this is to measure not only the uranium concentration in the working atmosphere, but also the physico-chemical characteristics of the pollutant :

1) the conditions under which the uranium compound was manufactured (temperature, purity, associated elements);
2) exact chemical nature (degree of oxidation, valency state);
3) conditions under which it was stored (humidity, light, etc.);
4) granulometry, particle density;
5) degree of enrichment (influence of the isotopic composition); and
6) degree of solubility in vitro in synthetic biological liquids.

All these characteristics must be known before individual radiotoxicological results can be interpreted. It is proposed to define the important elements to be taken systematically into account during any monitoring of workers exposed to uranium compounds. The objective aimed at is essentially a practical one : a check list ought to be filled in before any interpretation is effected. In this way, all characteristics of the workers' exposure will be available for the study of the metabolism of the incorporated uranium (second project).

2. Study of the metabolism of the incorporated uranium and of the health of the workers.

Accidental single and chronic or permanent contamination depending on the working place, the workers duration of service, etc., is quite frequently observed among the workers monitored.

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RADIATION PROTECTION PROGRAMME

Continuation contract no.: BIO-D-372-81-F

A detailed study will be made of the urinary excretion and faecal elimination of uranium and of uranium accumulation in the lungs. The various conditions of exposure will be taken into account. The study will be carried out in the following way :

a) Study of the urinary excretion of transferable, averagely transferable and non-transferable compounds

The majority (several thousand a year) of tests to determine the amount of uranium in urine will be performed on samples from a single urinic discharge,in which the amount of creatinine will also be determined in order to take account of the dilution or the concentration of the sample. In addition, tests for uranium content will be carried out on the urine and stools over a 24-hour period from 10 volunteer subjects (account being taken of the same parameters, the nature of the compounds and the content of the inhaled air).

b) Accumulation in the lungs

In a few months, an advanced anthropogammametry facility adapted for the measurement of uranium in both lungs will be available. The various results (urine, stools, lungs) will undergo a detailed statistical analysis in correlation with the parameters of exposure to the various types of pollutant measured jointly in the first project. It will therefore be possible to define the pattern of urinary excretion, faecal elimination and retention in the lungs of the various types of compounds studied. All the measurements of the urinary and faecal uranium on the one hand, and those carried out by means of anthropogammametry on the other hand, will make it possible to improve the knowledge of the behaviour of uranium through the organism.

c) Study of the workers health

A detailed medical survey, covering the various functions (pulmonary, renal, haematological, etc.) will be performed on a group of workers exposed in certain cases for many years to high concentrations of uranium-containing compounds. It is likely that the toxicity of uranium compounds is lower than that described in the literature.

When the various parameters measured elsewhere are available (conditions of exposure, urinary excretion, accumulation in the lungs), it will be possible to investigate and measure impact on the health of these workers, chronic ailments, deaths, etc., as a function of the duration of exposure, the work places occupied and the nature of the uranium compounds handled.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-371-81-F

General subject of the contract:

Genetic recombination as a mechanism of induction of leukemia upon irradiation and its impairment.

Description of research work:

The present research work proposed will be conducted as a fully collaborative project between INSERM, Unité 117 and CEN Mol. It is understood as the continuation of common efforts initiated several years ago and having yielded fruitful results.

It is well documented that X-radiation is an active leukemogenic agent for most mammalians including primates and humans. In the C57BL mouse it was shown that radiation-induced thymic lymphomas were associated to de novo expression of retroviruses. Although they were not well established as the direct cause of tumour induction, a number of observations favour such an hypothesis. In addition, it is now generally agreed that all the sarcomatogenic viruses described so far in the avian, feline, murine as well as in the primate systems and in most, if not all, the murine lymphocytic leukemia viruses are actual recombinants.

The INSERM group will develop more specifically the following points :

A. Possible hypotheses concerning the effects of X-radiation in C57BL mice;
   1. Genetic recombination between endogenous viruses, leading to the emergence of a leukemogenic neovirus (exogenous);
   2. Unmasking of endogenous proviral sequences and the influence of the re-insertion site(s) in DNA.
   3. Genetic recombination between endogenous (non leukemogenic), viral and cellular sequences and : (a) subsequent formation of leukemogenic and, possibly, defective virus ; or (b) action on the differentiation process of the lymphoid cell compartments.

B. Test for a recombinational event in ageing-related and in virus-induced leukemogenesis.
   1. Kinetics of expression of endogenous viruses (N and X-tropic) and their tissular localization.
   2. Modification of the kinetics of endogenous and recombinant viruses following inoculation, at various lifetimes, of the different viral components.
   3. Demonstration of the emergence of leukemogenic recombinant virus
      a) polymorphism of leukemias induced by the same viral isolate;
      b) modification of the latency period in a given leukemia
      c) exogenous nature of the virus,

d) serological and biochemical evidence for recombination :
   1. fingerprints of tryptic digests of viral proteins,
   2. fingerprints of RNases T1 digest of viral RNA,
   3. topography of proviral sequences in normal and in tumour DNA,
   4. demonstration of genome sequence specific of recombinant genome.

C. X-irradiation and expression of viral genes
   1. Kinetics of virus expression following treatment with leukemogenic sub-leukemogenic and supra-leukemogenic, X-ray doses in relation to age.
   2. Tissular localization of viral expression.
   3. Same as (1) and (2) after restoration with normal or irradiated bone marrow cells of thymectomized or intact mice.
   4. Leukemogenic potential of the various cellular compartments expressing virus following irradiation (bone marrow, spleen, thymus, lymph nodes, lymphocytes...).
   5. Demonstration of recombinational events :
      a) between endogenous viruses
      b) between endogenous viruses and cellular sequences (leading to the emergence of leukemogenic, possibly defective virus)
      c) importance of the reinsertion site in the host cell DNA.

D. Impairment of the leukemogenic process
   1. At the virus level : a) by raising antibodies against antigenic determinants specifically shared by the recombinant leukemogenic recombinant; b) by raising antibodies against otherwise non expressed endogenous viruses.
   2. At the cellular level : by grafting into irradiated mice, their own lymphoid cells which were collected and cryopreserved prior to the exposure.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-474-81-UK

General subject of the contract:

Non-stochastic effects of radiation on normal tissues relevant to radiotherapy and radiation protection.

Description of research work:

1. Cell kinetics and radiation damage. The relationship between tissue kinetics and radiation response is of basic and practical importance. Recently a refinement to the accepted concepts on the basis that tissues fall into two categories was suggested; i.e. H and F. In type H (hierarchical) there exists a defined stem cell compartment differentiating into histologically distinguishable functioning cells. Radiation-produced tissue damage will result from inadequacy in the mature cell compartment which depends on the lifespan of these cells. A similar model has been the accepted view for all tissues for many years. In type F (flexible) tissues all cells are assumed to be capable of both proliferation and also of performing tissue specific functions. Radiation will lead to dose-dependent loss of functional cells through their mitotic death, both following exposure and during the next phase of increased compensatory proliferation, resulting in accelerated expression of radiation damage ("avalanche"). Consequently the more severe damage following larger doses of radiation is seen earlier than the milder reactions produced with smaller doses. Likely examples of type H tissues include epidermis and intestinal epithelium, and type F tissues dermis, endothelium, neuroglia and liver parenchyma. The differences between tissue types H and F have important clinical implications. Types H tissues respond at a time characteristic of the tissue and largely not dependent on the dose. After this time it is unlikely that any further consequence of irradiation will ensue. In contrast, the response of type F tissues occurs later with increasing dose so that the possibility of serious late consequences can never be excluded. In addition, type F tissues are likely to respond dramatically to some unrelated trauma, e.g. that due to an infection or mechanical injury. Such recall phenomena are well known in radiotherapy.

Support for this concept exists in the literature, but is not adequate. It is proposed to construct mathematical models of these two types of tissue and predict the response using computer simulation techniques. Comparison will be made with the results of animal experiments. These will include measurement of the timing of radiation response and its modifications by subsequent trauma, e.g. contralateral nephrectomy at long times after irradiation of one kidney. Single doses of X-rays and fast neutrons will be used and followed by pulse, pluse-chase and continuous tritium-thymidine labelling in vivo at different times after

exposure. Quantitative analysis of the microautoradiograms will provide data on tissue kinetics during the radiation-induced depopulation leading to functional impairment. Tissues thought to be type F will be given most attention, e.g. thyroid, kidney and non-epithelial components of intestine. Use will be made of new methods of assessing radiation injury, such as measurement of the intestinal absorptive surface in histological slides of jejunum, with development for measurement of late injury.

2. Vascular injury and late radiation damage. Late radiation injury may be related to the proliferation kinetics of tissue parenchymal cells as discussed in project I. An alternative hypothesis is that radiation-induced changes in blood vessels lead to death of dependent parenchymal cells. Increases in vascular permeability may lead to fibrosis in the wall of small blood vessels and in the tissue spaces, leading to a reduction in vascular perfusion and a further tissue response. However, the precise role of the vascular system in radiation damage is not clear. To study vascular damage after irradiation with X-rays or fast neutrons and to investigate its role in late tissue reactions it is proposed to make serial measurements of anatomical and physiological changes in both blood vessels and parenchyma of the kidney and lung. The relationship of these changes to late fibrosis will also be investigated.

Non-invasive techniques will be developed for measuring vascular function in rats. Short-lived radioisotopes, produced by the MRC Cyclotron, with visualisation by means of a new positron camera (spatial resolution 6 mm fwhm), developed by the Rutherford Laboratory, will be used. Vascular permeability (e.g. IV injection of Ga-68 transferrin), blood volume (e.g. red cells labelled by inhalation of C-11 monooxide), blood flow (e.g. inhaled C-15 dioxide), oxygen uptake (inhaled oxygen-15), kidney function (e.g. F-18-hippuran) and distribution of alveolar surfactant (injection of C-11-palmitate) will be measured.

Histological changes in small blood vessels will be quantitated by a method which has been developed for rodent tumours. Vessels containing red cells are visualised by staining haemoglobin with benzidine. Cleared tissue is mounted in perspex blocks and vascular changes quantitated by drawing method. It is proposed to extend this technique using an image analyser.

Late fibrosis will be quantitated by measuring hydroxyproline, which is present in collagen but not in other mammalian proteins. Collagen synthesis will be investigated by determining the incorporation of radioactively labelled proline (as hydroxyproline) into collagen.

Wherever possible these techniques will be applied to extended fractionation procedures with both neutrons and X-rays.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-366-81-D

General subject of the contract:

Pathogenesis of late somatic effects of radiation.

Description of research work:

The research programme is a continuation of past and current work on the pathogenesis of late somatic effects of radiation. The major theme will be again the study of late effects of incorporated radionuclides. The experimental work is concerned with three important problems:
1. The first main topic is the evaluation of the risk of induction of late effects, especially bone tumours, which may result from exposure to bone-seeking radionuclides, with special emphasis on the time factor and radiation quality. In addition to the time factor experiments with multiple exposures to short-lived alpha- and beta-emitting radionuclides, which have already been carried out, more complex dose-time patterns will be studied. The relative risks of induction of bone and soft tissue tumours, or of leukaemia by the combined exposure to very low levels of long-lived and different dose levels of short-lived radionuclides or external irradiation will be investigated.
2. A problem of particular interest is in the context of this research the pathogenesis of late effects after internal irradiation. A continuation and expansion of the experiments on radiation oncogenesis in bone is intended with the aim of providing answers to certain specific questions concerning the basic mechanisms by which internally deposited alpha-emitting radionuclides induce bone tumours. Protracted irradiation of mice with the short-lived alpha-emitting Ra-224 (1100 rads over 36 weeks) results in an osteosarcoma incidence of nearly 100% in a rather well synchronized latency time. This provides an excellent experimental tumour model for this type of studies. The knowledge of the processes and stages of carcinogenesis is mainly based on experiments with the induction of epithelial tumours. The objective will now be, to evaluate the process of oncogenesis in the skeletal tissue. Initially these studies will mainly be concerned with early stages of tumour development and preneoplastic lesions, and with the question whether bone tumour development may be a multi-stage process. Using the same tumour model the experiments on C-type retrovirus expression will be extended. More data on virus expression and antiviral antibodies during the osteosarcoma latency period, and in osteosarcoma bearing animals are needed, to use these parameters as possible indicators or predictors of radiation-induced osteosarcoma development. In addition specific immuno-histochemical studies using viral antigens or antibodies, will be developed, in order to trace transformed cells in situ. This will be a new approach for the

evaluation of cells at risk and of the first steps of oncogenesis.
Finally one may use this bone tumour model to study the growth pattern and cell kinetics in morphologically different types of radiation-induced bone tumours. These data may become of interest with regard to the therapy of osteosarcomas.
3. The third main topic of research will be devoted to the question, to what extent are the risks of radiation-induced bone or other tumours modified by various endogenous and exogenous factors. The endogenous biological factors will include in addition to age and sex disposition species or strain susceptibility and naturally occurring diseases. Estimation of osteosarcoma risk at low dose level must consider the influence of the genetic background of susceptibility. There are genetic links between the histocompatibility system and loci of certain isoenzyme and endogenous viruses and spontaneous tumour risks. This genetic information can be used in experiments comparing different strains of mice which are genetically different and/or have a different spontaneous pathology. In repeated experiments with the same strains of mice the possibilities of genetic monitoring techniques will be studied.
The exogenous factors will include experimentally induced diseases using established animal models of disease or additional exposure to other environmental contaminants and drugs, especially those, which may influence the metabolism or proliferative capacities of the tissue or cells at risk, or which will modify the tumour-host relationship and immunological conditions during the latency period.
In this project are planned for the future, in addition to the bone tumour experiments, studies of synergistic effects with the combination of internal radiation and chemicals in hepato-carcinogenesis and morphological studies of possible synergistic non-stochastic late effects using the eye as target organ.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Prof. Dr. A.M. Kellerer*
Prof. Dr. H. Spiess*

Contract no.: BIO-D-461-81-D

General subject of the contract:

Epidemiological studies of the radiation carcinogenesis and its biophysical basis.

Description of research work:

Radiation carcinogenesis is the major factor that contributes to the risks of small doses of ionizing radiations. Human epidemiological studies, as well as animal experiments are required for its elucidation. The assessment of human data is subject to considerable difficulties that range from the limitations of dosimetric estimates, and the incompleteness and loss of information in follow-up studies to the restricted number of individuals in the groups under study. The selection of meaningful and representative control groups is also a critical and often difficile task. In spite of these problems human studies are required and essential; and without them the extrapolation from animal studies would remain tenuous and entirely unreliable.
The epidemiological studies in project 1 and 2 are closely linked as they deal both predominantly with follow-up studies in 224-radium patients. The study of bone seeking -emitters is of particular pragmatic importance, and the past investigation on the 224-radium patients has with comparatively modest means led to very substantial insights, particularly on the induction of osteosarkomas. The continuation and extension of these studies is therefore of greatest importance.
As the human epidemiological studies provide a firm but numerically only approximate basis for risk estimates they have to be complemented by animal experimentation. This extension alone can provide data in the actual dose range relevant to radiation protection. The linkage of different human epidemiological studies with each other and with experiences from animal experiments will, however, require greater efforts to utilize coherent and rigorous statistical techniques. This is a particularly important objective of project 3 that aims at establishing closer interrelation between the radiation carcinogenesis studies in the CEC Radiation Protection Programme and also those in a number of laboratories in the United States. Development of more coherent statistical and epidemiological techniques and adaptation of existing statistical methods for the analysis of radiation carcinogenesis will be the essential aim.

*This research programme is carried out in coordination with the "Institut für Medizinische Strahlenkunde der Universität Würzburg", Prof. Dr. A.M. Kellerer and the "Kinderpoliklinik der Universität München", Prof. Dr. H. Spiess.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
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Contract no.: BIO-D-301-81-NL

General subject of the contract:

Dosimetric and experimental studies on long-tumour induction by inhalation of radon.

Description of research work:

Research will be executed by the contractor concerning radiation hazard of inhaled radon, expecially with regard to tumour induction.
Radioactive nuclides, such as uranium and daughter products, have always been present in the natural environment of man. Variations in this natural background of ionizing radiation related to location in different countries or altitude have never been demonstrated to result in a significant shortening of life span or specific types of cancer incidence.
Technical innovations in the beginning of this century requiring large amounts of uranium ores, resulted in a significant shortening of the mean life span of the uranium miners in comparison to that of the general population. This life shortening was demonstrated to be due to an enhanced lung-cancer-frequency in this group of workers. This increase could be related to the increase in lung dose of alpha particles from radon daughters.
From the epidemiological studies on these uranium miners, an RBE factor of about 5 for the radon daughters was deduced. This value for the RBE factor is rather low in comparison to the quality factor of 20 for alpha particles adopted by the ICRP. Calculations of the risk factor by the ICRP task group on Biological Effects of Inhaled Radionuclides on experimental animal data tends to support a quality factor of 20.
The reported differences in risk coefficients might be related to uncertainties in dosimetry as well as to uncertainties in the deposition pattern of the radioactive aerosols over the lung region. This deposition pattern will depend on the size of the various structures of the lung, which change with age of the individuals.
The difference in the quality factors are, apart from health-physics aspects for miners, of interest for the general population of industrialized contries. In these countries there is a mounting application of gypsum, originating as a waste product of the aluminium and phosphate industries, for building material. The increased radium content of these materials in combination with the reduced ventilation in private houses (resulting from conservation measures) may pose a potential health hazard. For a correct evaluation of this potential better insight in the various factors determining risks is required.
Experimental tumour induction studies for risk analysis are generally performed over a relatively short period of the animal life span and

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RADIATION PROTECTION PROGRAMME

Continuation contract no.: BIO-D-301-81-NL

for high concentrations of radon. Moreover, inhalation conditions in these studies were aimed at simulating the dust particles composition comparable with mining conditions. Therefore, studies with other conditions are important for the evaluation of risks in houses.
In this research programme, theoretical and experimental studies will be combined to evaluate the potential risk for cancer induction by inhaled radon. The theoretical studies will involve calculations of energy deposition by alpha particles in various structures of the lungs in dependence on the size of the bronchi and alveoli and in relation to physical parameters (such as : distribution of radioactive aerosols, fraction of unattached radon, radon-daughters equilibrium conditions). These calculations are important to correlate data on young and adult rats, as well as for the extrapolations of data on rat to man.
In the experimental study, WAG/Rij rats, starting at an age of 4 to 6 weeks, will be exposed daily for periods of about 18 months (1/4 of their life span) in an exposure chamber to a relatively low concentration of conditioned radon air dust mixture. Total doses will be given up to 500 WLM. The lungs of these rats will be analysed for lesions after spontaneous death and after sacrifice as required by the health conditions of the animals.
Collaboration and coordination with the CEA, Fontenay-aux-Roses (Prof. Dr. Lafuma) will be established.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-382-81-UK

General subject of the contract:

Pathogenesis of early and late radiation reactions in normal tissues.

Description of research work:

When normal tissues are exposed to radiation as a result of an industrial accident or clinical exposure, non-stochastic changes are produced if threshold doses are exceeded. Such radiation injuries may lead to unacceptable morbidity or mortality. It is proposed to examine the early and late effects of both single and fractionated doses of radiation on the pig skin and the vasculature of the hamster cheek pouch. Attention will be paid to the mechanisms of radiation effects with specific reference to changes in the vascular system and epithelial or parenchymal cells. In the skin particular attention will be given to the interaction of vascular and epithelial elements in the process of wound healing.

In the first project, early and late effects of irradiation in pig skin will be studied. Pig skin which has a similar histological appearance and vascular structure to that of man, provides the best animal model in which to investigate radiation effects, if the results obtained are to be extrapolated to man. In the acute reaction, the independent assessment of erythema, pigmentation, desquamation and dermal necrosis will continue to be used to evaluate the complex interaction of factors responsible for the early reaction of skin to radiation. It is planned to establish minimum dose levels consistent with the development of desquamation and dermal necrosis for a range of irradiation regimes of 250kV x-rays.

Studies with x-rays will continue to investigate the detailed mechanisms responsible for the acute reaction. Attention will be given to changes in epithelial cell number and kinetics when small daily doses are given over 6 weeks. Such studies will be extended to examine epidermal reactions after longer periods of daily fractionation.

We will continue to examine changes in the dermal and subcutaneous vascular system. The important reduction in blood flow 3 - 4 months after irradiation will be examined by a comparison of blood flow and histological changes. The role of vascular damage in the development of late radiation atrophy will be investigated.

In the second project, the biological effects of non-uniform irradiation will be investigated. In operational procedures involving radioactive material, the limiting dose is often that to the skin. Exposures are rarely uniform and could involve that from very small

particles in contact with the skin. As non-stochastic effects (desquamation, ulceration, atrophy etc.) should be prevented, there is a need to investigate the early and late effects of irradiation with sources of different size, for a range of emission energies. Such investigations will be undertaken on pig skin based on the methodology and experience gained from our investigations into the effects of uniform irradiation in the skin. The depth of the basal layer and thickness of the dermis in the pig are similar to man ; thus the results can be used for the establishment of protection criteria.
The main contribution to the skin dose is likely to be from beta irradiation and so the nuclides, Promethium-147, Thallium-204, Thulium-170 and Strontium-90 have been selected for investigation. They have a beta-range in tissue of 0.3; 2.2; 3.2 and 8.0mm respectively. The source are circular, with diameters of approximately 1-40mm. As well as providing a basis for the development of criteria for the non-uniform irradiation of skin, the results may help to elucidate some of the basic biological mechanisms involved in the reaction of skin to radiation.
The third project studies the healing of an acute radiation burn or late necrosis of the skin as influenced by damage to the surrounding vascular connective tissue. This damage will also be a limiting factor in the success of any conservative treatment involving reconstructive surgery. However, little is konwn about the healing process in skin when the vasculature is damaged by radiation. It is proposed to evaluate some of the problems involved using free skin grafts in the pig. The rate of wound healing will be determined by establishing the time taken for the revascularisation of grafted skin. Improvements in vascularity will be measured in terms of vascular function in the graft. The results for normal skin will be compared with those in which the grafted area has been irradiated. The effects of irradiation dose and time between exposures and grafting, on the wound healing process will be investigated.
Project four will investigate the pathogenesis of late vascular damage. While experiments in the skin, central nervous system and other tissue may indicate how vascular changes contribute and interact with parenchymal elements in the development of late effects, there is still a need to investigate vascular changes in a simpler system. Thus, investigation into the effects of radiation on the fine vasculature of the hamster cheek pouch will continue. This structure can be everted, allowing repeated measurements of vascular density and vessel diameters. A cellular basis for late vascular lesions will be a priority, as little information exists on radiation effects on the various cell types in the blood vessel wall.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. S. Hornsey
Cyclotron unit
MRC
Ducane Road
GB-London W12 OHS

Contract no.: BIO-D-442-81-UK

General subject of the contract:

Radiation damage to cells and tissues at dose levels relevant to radiotherapy and protection.

Description of research work:

There are indications that patients are being overdosed when tumours located in areas intimately associated with nervous tissues are irradiated with neutrons (e.g. gliomas, cervical nodes). Work in this department to measure the sensitivity of the spinal cord and brain and other published data indicate that the CNS tissue is relatively resistant to photon radiation because of the large capacity to repair sublethal damage. High RBE for neutrons have been obtained for the spinal cord. That these values also apply to brain damage needs verification. Neutron irradiation of rat brain will be extended to multifractions so that RBE in the therapy range of doses may be determined. Repair in brain and spinal cord will be investigated.

Because of the normal considerable capacity to repair sublethal damage observed in the CNS, markedly increased radiation sensitivity may be associated with degenerative diseases. It is proposed to investigate this postulate using strains of animals with demyelinating and degenerative nerve diseases. The sensitivity of these animals to radiation, both in nervous and other tissues, will be compared with that of normal animals to investigate the relationship between these degenerative conditions and repair deficiency. Sensitivity will be investigated by observing the onset and incidence of radiation induced paralysis and also by measurement of nerve conduction velocities.

Skin sensitivity will also be observed. In man some radiosensitive repair deficient mutant cells have been obtained from the skin of patients with degenerative disease of the CNS, e.g. ataxia telangectasia and Huntington's chorea. Some of these mutations affect radiation sensitivity even in the heterozygote and may account for some of the variation in sensitivity in "normal" human populations. The present investigation will include fractionation studies going to dose levels which may have implications for "nonstochastic" radiation hazards particularly in populations which may carry repair deficient mutations.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. Dr. H. Kriegel
Abt. für Nuklearbiologie
GSF
Ingoldstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-D-365-81-D

General subject of the contract:

Decorporation and interruption of transfer of radionuclides; especially of radioactive alkalines and alkaline earthes.

Description of research work:

Three main directions will be followed to study decorporation of alkaline earthes :

1) Decontamination and transfer interruption of radiostrontium, radio-barium and radium on the intestinal level by a recently developed 222-derivative.

a) Experiments in mammals, which will demonstrate the inhibitory effect of the 222-derivative on the transfer of Sr-85, Ba-133 and Ra-224 to the skeleton.

b) Experiments in pregnant mammals, which will demonstrate the inhibitory effect of the 222-derivative on the diaplacental transfer of Sr-85, Ba-133 and Ra-224 to the fetus.

c) Experiments in lactating mammals, which will demonstrate the inhibitory effect of the 222-derivative on the transfer from food to milk and litter.

2) Transfer inhibition or impairment of circulating radioalkaline earthes to bone.

a) Sr-85 decorporation studies concerning the dose-time effect relationship with cryptand 222 in mammals bigger than rats by a well developed kinetic model.

b) Theoretical studies with cryptant 222 and related compounds concerning an optimization of the tentatively developed treatment scheme for Sr-85, Ba-133 and Ra-224 contaminated man.

As in the past the project will include chemical work in order to develop new agents respectively alterations of the used molecules. These studies will be limited to the frame of the proposed project.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. J. Lafuma
Serv. Radiopath. et Toxicol. Exp.
CEA-CEN de Fontenay-aux-Roses
B.P. n°6
F-92260 Fontenay-aux-Roses

Contract no.: BIO-D-370-81-F

General subject of the contract:

Study of the biology of the Pu-tributylphosphate complex, metabolism and possible decorporation.

Description of research work:

In the separation of plutonium-239 by the PUREX process, one of the stages requires the plutonium to be complexed with tributyl phosphate (TBP). This complex is stable in a highly acidic medium and soluble in fats. The problem is whether at the moment of neutralization, which occurs on contact with living matter, the complex, is destroyed before being incorporated in body lipids. Preliminary experiments performed on rodents have indicated that the "in-vivo" behaviour of the Pu/TBP complex is completely different from that of inorganic plutonium compounds. The metabolic process after inhalation or after intramuscular injection simulating wounds, is quite different from that of plutonium citrate or nitrate, inasmuch as :

1. diffusion is more rapid;
2. faecal excretion is greater.

These findings are consistent with certain data obtained in the case of human contamination, which have given rise to problems in calculating body burdens owing to :

3. a high burden in the bones;
4. a low ratio of urinary activity to bone activity.

Therapeutic tests with DTPA have produced no result.
On the other hand, the behaviour of the Pu/TBP complex after oral absorption is comparable to that of plutonium-IV salts.
The programme is planned for four years and will involve baboons (papio papio). It will consist of three parts :

1. Complete metabolic studies after inhalation on intramuscular injections simulating wounds.
   The studies will cover :
   - "in-vivo" measurements of the evolution in local retention
   - excretory processes : urine and faeces
   - distribution in the body at the times of saçrifice after 3 days, 15 days, 1 month, 3 months and 1 year respectively.

   Each event will involve two animals, i.e., 10 baboons for each of the modes of contamination. A total of 20 baboons will be used and the experiments will be spread over two years.
2. Effects of pulmonary washing.
   This study, to last one year, will involve 10 animals. The washing sessions will be held at time intervals fixed after the earlier studies on inhaled plutonium oxides. The efficacy of various washing

liquids will be compared namely a solution of NaCl, with or without the addition of a chelating agent or solvents capable of entraining the TBP.

The measuring methods will be the same as those used in the first part of the research.

3. Effects of chelating agents administered by injection.

This study, to last two years, will use 10 baboons contaminated by intramuscular routes or by inhalation. It will use the same methods as the two previous studies.

DTPA and in particular, if available, the new chelating agents synthesized at the NRPB (e.g., Puchel) will be used as the chelating agents.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-D-383-81-UK

Prof. P.J. Lindop
Dept. Radiobiology
St. Bartholomew's Hospital
Charterhouse Square
GB-London EC1M 6BQ

General subject of the contract:

Radiation damage from inhaled particles in the rodent lung

Description of research work:

This is a collaborative project between the Radiobiology Department, St Bartholomew's Hospital Medical College, and the Inhalation Toxicology Group of the Division of Environmental and Medical Sciences, AERE Harwell. The aim of the research is to extend the work carried out under the previous contract in this field. That contract was concerned with the administration of sized aerosols of plutonium-239 dioxide to mice and the study of short and long term effects in an attempt to relate spatial distribution of dose to effects in the lung. This study has shown that. even at the lowest initial lung burden used (0.1 nCi), there was a significant increase in the incidence of pulmonary tumours and at higher lung burdens very considerable life-shortening as fibrosis became important in the impairment of lung function. Following on from this work and because the pattern of exposure of workers is unlikely to involve just one intake it is proposed, also, to simulate this by exposing mice to a semi-maintained lung burden of plutonium-239 dioxide and to study the effects of this on the lung. The work under the existing contract has shown a decrease in the numbers of alveolar macrophages following inhalation of plutonium-239 dioxide particles followed by recovery to normal levels. Thus, under conditions where a maintained lung burden is established by repeated exposure, the recovery of the lung's macrophage defence system may be studied. It is proposed, therefore, to re-expose mice at about two monthly intervals to maintain a lung burden less than that which produced a peak incidence of tumours in our initial single exposure work (say, 0.5 nCi). The repeated exposures will be over a period of about 1 year and be applied to mice starting at ages of 6 weeks to about 6 months (in an attempt to simulate differences in age of humans). Initially, the study will assess the effects on alveolar macrophage numbers and the incidence of neoplasia at 1 year following the first exposure of a single maintained lung burden. Eventually it is intended to extend the study to include a range of exposures, and challenges to further test and investigate the lung's defence system.

It is further intended to study the long and short term effects of the inhalation of Am-241 aerosols in mice. This has a practical basis in that the hazard in a nuclear power industry using high burn-up times of fuel comes as much from Am or Cm as from Pu. Also, it is to be expected that Am will produce a more homogeneous dose than translocation to the

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Continuation contract no.: BIO-D-383-81-UK

lung tissue, thus providing a contrast and means of comparison with the work already completed with plutonium-239 dioxide. In addition, Am will be translocated from the lung much faster than Pu. Because of this speed of translocation it will be necessary to study the deposition and effects produced in other tissues with particular emphasis on pulmonary lymph nodes, liver and bone - the use of the mouse will be an added advantage in this. This study will only encompass a polydisperse aerosol of Am-241 (probably nitrate) and a limited range of initial lung burdens.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. P. J. Lindop
Dept. Radiobiology
St. Bartholomew's Hospital
Charterhouse Square
GB-London EC1M 6BQ

Contract no.: BIO-D-447-81-UK

General subject of the contract:

Radiation damage from inhaled particles in the rodent lung

Description of research work:

The previous contract was concerned with long and short term effects of the inhalation of Pu-239 dioxide particles by mice in relation to the spatial distribution of dose. The use of three sized aerosols (0.7 μm AMAD, 1.4 μm AMAD and 2.1 μm AMAD) was proposed and so far groups of mice have been exposed to the large and intermediate sized particles at several different initial lung burdens. Exposures to the smallest particle size aerosol will take place in 1980.

An important part of this project has been the study of the translocation of Pu from the lung to other tissues both quantitatively, in terms of rate and amount of Pu accumulating, and qualitatively, in terms of pathology resulting from the translocated material. These studies involving the measurement of Pu in a number of tissues at low level have been time consuming and expensive but will need to continue because a definite dependence has been found of the rate of removal and redistribution from lung on the size of the initial lung deposit. This will be of importance when considering extrapolation to man and the concept of total risk. It is also of great importance in the estimation of average alpha dose both to the lungs and other tissues particularly when comparison is required with the effect of other forms of irradiation. This work will be further extended to low levels by the utilisation of novel methods of autoradiography with CR39 plastic. Using these techniques low level translocation to extra pulmonary tissues will be measured, the alpha distribution mapped and related to pathology developed, e.g. in the lymph nodes, liver and different bones. The material available from the mice exposed so far provides a unique opportunity for such a study.

In addition to metabolic experiments the major study has been to assess quantitatively and qualitatively the pathological damage produced both in the lung and elsewhere. This has been divided into two parts :

(1) Pathology, particularly in the lung, assessed at 1 year after inhalation
(2) Long term survival and detailed pathology at death assesed in all other mice

The methods, which will continue to be employed involve a lung "clearing" technique, which reveals tumours or nodules at a very early

stage followed by detailed histology and histopathological investigation of all other major organs and tissues. Obviously rigorous studies of this nature are again time consuming and costly but are necessary to determine the dependence of tumour incidences in extra-pulmonary tissues (due for instance, on the depression of immune surveillance) on the lung Pu content. A great deal of material has already been preserved and some histology assessed but the greater part of this work will be involved in this 1981-1982 research programme.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. J. R. Maisin
Dép. Radiobiologie
CEN/SCK
Boeretang 200
B-2400 Mol

Contract no.: BIO-D-378-81-B

General subject of the contract:

Late somatic and genetic effect of radiation in mammals.

Description of research work:

Project one studies the morphological, biochemical and physiological late effects of irradiation in the adult and developing rat brain. Studies on the irradiated brain of adult animals showed alterations in the glia population after doses much lower than those required to cause vascular damage or a shortening in life span. Time periods up to 27 months will be included and studies on the cell kinetics of the glia will be done to elucidate the mechanism of the changes observed. The work on the developing brain will be carried out from birth up to 24 months after an irradiation with 0.1 to 1 Gy carried out either prior implantation during organogenesis or late in pregnancy. The late effects of radiation on the embryo will be assessed on the basis of density, distribution and structural lesions of different glia cells, thickness of the cortex, vascular density and lesions and permeability of the blood brain barrier to peroxydase. The biochemical properties of isolated endothelial fragments of adult brain and the regional blood flow in relation to total blood flow and vascular architecture will be investigated. Studies on developing brain (identical conditions as for the morphology) will deal with the biochemical maturation of the brain-development of biogenic amines, of lysosomal enzymes, of lipids related to myelination - as well as with alterations in endothelial functions and blood flow.

Project two studies the effects of whole body irradiation of the developing organism on the hematopoietic system and on life span and disease incidence. The acute effects on the number of stem cells in spleen, bone marrow and liver, their in vitro radiosensitivity, as well as the late development of alterations in the regenerative ability of stem cells will be investigated after irradiation during the late part of pregnancy and early after birth. Simultaneously, groups of animals will be followed for their entire life span and autopsied at the time of death to establish the causes of death. These experiments will also be carried out with fractionated exposure.

Project three considers the mechanism of radiation induced fibrosis in lung. Previous studies on lung have indicated that the metabolism of connective tissue components, of lipids, of blood clotting factors and the blood flow are already altered before fibrosis becomes manifest. The future work will follow these leads and characterize in more detail these alterations. Specific functions of the lung such as degradation of prostaglandins and biogenic amines, conversion of angiotensin, the

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Continuation contract no.: BIO-D-378-81-B

metabolism of connective tissue components and the behaviour of the fibrinolytic system will be studied at different times after irradiation of the lung. A knowledge of the mechanism by wich radiation-induced fibrosis originates may eventually indicate means for its prevention, and agents which may act on the fibrotic development will be tested on the lung system. Organs from continuously irradiated dogs will be obtained from Argonne (USA) to assay composition of different connective tissue components in relation to morphology in order to investigate which role fibrosis plays in ageing and non cancerous disease under these conditions. The influence of dose rate and fractionation on life shortening and causes of death of mice irradiated with 50 MeV neutrons and gamma rays are studied in project four. During the past contract, the RBE of neutrons was compared to gamma rays with respect to the induction of leukemia and tumours and to the shortening of the life span, using various fractionation schedules, radioprotectors and animal strains with different disease characteristics. This study will now be extended to short fractionation intervals (in the order of hours). Such experiments will, be carried out in C57B1 and BALB/c mice selected for their differences in spontaneous rates of leukemia and other cancers and will involve doses from 0.02 to 2 Gy of neutrons and from 0.25 to 4 Gy of Co-60 gamma rays. Project five consideres the factors influencing genetic radiosensitibility and their influence on the extrapolation of genetic data from animals to man. Knowledge of the different factors that determine the relative yield of radiation-induced genetic alterations in different species is indispensable for an extrapolation of animal data to man. Recent investigations, using the Harlequin technique, have shown that some of the interspecies differences reported earlier for cultured lymphocytes result from an elimination of damaged cells during the subsequent divisions. Several species will, therefore, be compared with respect to the yield of aberrations observed in the various divisions following irradiation of lymphocytes. Any remaining interspecies differences will then be evaluated with respect to the other factors cited above. Most experimental animals have a much shorter lymphocyte life span than man so that aberrations are more readily eliminated in the former ones. Studies on lymphocyte lifespan and elimination of radiation induced aberrations will be carried out in several species, in particular in the dog. The influence of dose and inhomogeneous exposure on the aberration yield in radiotherapy patients is considerd on project six. An increased yield of aberrations has been observed after accidental generalized irradiation, but information on the effects of inhomogeneous and fractionated exposure is scanty, aside from data on aberrations in patients treated for ankylosing spondylitis. The influence of dose distribution on aberration yield in lymphocytes will be studied, in cooperation with a radiotherapy unit, by determining before and during the therapy the yield of aberrations in cultured lymphocytes. The tumours will be chosen to give radiation fields of different characteristics so that the influence of tissue volume and organ involved can be discerned.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. J. R. Maisin
Dép. Radiobiologie
CEN/SCK
Boeretang 200
B-2400 Mol

Contract no.: BIO-D-379-81-B

General subject of the contract:

Genetic recombination as a mechanism of induction of leukemia upon irradiation and its impairment.

Description of research work:

The present research proposal has been drawn and will be conducted as a fully collaborative project between INSERM and CEN. It is understood as a continuation of common efforts initiated several years ago and having yielded fruitful results. The details of more specific roles of each laboratory will be found on their respective application form.

It is a well documented phenomenon that irradiation induces leukemogenesis. In the C57Bl mouse, it was shown that, in some instances, the thymic lymphomas were associated to de novo expression of retroviruses, including recombinant virus. Although they were not well established as the direct cause of tumour induction, a number of related systems favor such a hypothesis. The best documented are the MCF recombinant viruses which are specifically responsible for spontaneous thymic lymphomas in AKR mice ; all the sarcomatogenic viruses described so far (in the avian, feline, murine, as well as in the primate systems); and most, if not all the murine lymphocytic leukemia viruses.

The research proposal summarized below is designed to test the hypothesis of a recombinational event in the induction of leukemogenesis by irradiation, and to eventually develop a methodology for its impairment. The investigations at the CEN will deal with :

A. Irradiation, virus recombination and virus expression
   1. Emergence of leukemogenic virus as a consequence of irradiation; assessing their possible recombinant nature.
   2. Appearance of novel proviral sequences in relation with radiation induced leukemogenesis; assessing their possible recombinant origin and investigating a possible relationship between their insertion site in host cell DNA and the type of leukemia induced.
   3. Search for transformation-specific RNA and protein products in radiogenic lymphomas and lymphoma cell lines.

B. Mechanism of the induction of a transforming gene following viral infection.
   1. Insertion site(s) of newly acquired provirus after exogenous infection and relationship with the type of leukemia induced; comparison with radiogenic leukemogenesis.

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2. Investigating whether the leukemogenic potential of various leukemogenic virus isolates can be attributed to either their recombinant nature or rather to the activation of a cellular transforming gene by insertion of a viral promotor in its neighborhoud.
3. Determining whether the activated transforming genes are part or not of the proviral genomes.
4. Search for transformation -specific RNA and protein products in virus-induced lymphomas and lymphoma cell lines. Comparison with radiogenic lymphomas.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. A. Morgan
Env.and Med.Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

Contract no.: BIO-D-386-81-UK

General subject of the contract:

The effect of firing temperature on the solubility in lung of Pu-238 dioxide and Pu-239 dioxide; implications in biological monitoring.

Description of research work:

The main objective of this research programme is to investigate the effect of firing temperature on the retention of both Pu-238 dioxide and Pu-239 dioxide in the lung . A detailed description of the proposed research work is given below :

a) Samples of Pu-238 dioxide and Pu-239 dioxide will be prepared by calcination of the oxalates at a range of temperatures from 500-1500°C. This work will be carried out by the Chemistry Division at AERE, Harwell. Representative samples of airborne dust from fast reactor fuel fabrication facilities will also be obtained from AEE, Winfrith.

b) From these materials, samples with an AMAD of about 1.5 μm will be obtained by water sedimentation. Techniques for doing this have already been developed. This size of particle is not untypical of those encountered in nuclear facilities of the UKAEA and BNFL and is close to the optimum for alveolar deposition in rodents.

c) Samples of the sized materials will be administered to mice by inhalation and probably also to rats by instillation. In the mouse, it is planned to give an initial alveolar deposition of 1 nCi (37 Bq) which will not affect the lifespan of the animals significantly.

d) Groups of animals will be killed at various times up to 2 years post administration. The amount of Pu-238 and Pu-239 remaining in the lungs and transferred to other tissues (lymph nodes, bone, liver, kidney and spleen) will be determined. Analytical procedures for the determination of Pu-239 in tissue samples have already been developed.

e) Studies of a case of accidental exposure to Pu-238 dioxide have shown that DTPA, given intravenously, has been effective in reducing the lung burden. As a possible adjunct to the proposed programme, a study may be undertaken of the relative efficacy of DTPA given by inhalation and intravenously, in reducing lung burdens of plutonium oxides fired at different temperatures.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. A. Morgan
Env. and Med. Sciences Div.
AERE
Harwell, Didcot
GB-Oxon OX11 ORA

Contract no.: BIO-D-448-81-UK

General subject of the contract:

The induction by actinides of non-neoplastic changes in rodent lung.

Description of research work:

This is a continuation and extension of an existing research programme between the Inhalation Toxicology Group, Environmental and Medical Sciences Division, AERE, Harwell and the Dept. of Radiobiology, Medical College of St Bartholomew's Hospital. In this collaboration, the Harwell Group are studying the acute and sub-acute effect of plutonium-239 dioxide on the alveolar macrophage population of the mouse lung and the effect of particle size and dose on the induction and progression of pulmonary fibrosis. The Barts Group are studying the late-effects of plutonium-239 dioxide. Each laboratory is seeking support, separately, to complete work already started in the present programme. Proposals covering the various aspects of the AERE programme are outlined below :

1) Studies on the alveolar macrophage

Studies have been made of the short-term somatic effects of inhaled plutonium-239 dioxide on the rodent lung. Using a technique of in situ broncho-pulmonary lavage, the response of the alveolar macrophage to plutonium-239 dioxide has been investigated in the mouse. However, associated biochemical studies of cell changes have been complicated by the small sample size and contamination from the intact pulmonary vasculature. Therefore, the completion of this investigation will be undertaken using the rat. The cellular and biochemical response of the rat AM to non-radioactive mineral dusts have been studied extensively in this Department. Using many of the techniques developed for that programme, rats will be given doses of plutonium-239 dioxide sufficient to induce medium and long-term changes in the lung. Studies will be made of the effects on the AM population, of the associated leakage of cytoplasmic constituents into the airways and on biochemical parameters in the whole lung. These studies will be aimed at determining the nature of the damage as well as the regenerative capacity of various cell populations in the lung, as indicated in the mouse.

2) Studies on the fibrogenicity of actinides

The administration of various doses and sizes of plutonium-239 dioxide to mice, for an assessment of the fibrogenic response, was completed in 1980. The biochemical and histological assessment of tissues arising from mice exposed in this programme will extend through 1981. These studies are designed to define the relationship between fibrogenicity

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and the distribution of radiological dose in the lung. To complete this series, the fibrogenicity of 'soluble' americium-241 trinitrate will be compared to plutonium-239 dioxide at equivalent radiological doses to the lung. The mice will receive small, repeated exposures of Am-241 to compensate for its more rapid clearance from the lung.

3) Metabolism of Pu-239

The original contract was concerned with long and short-term effects of the inhalation of plutonium-239 dioxide particles by mice in relation to the spatial distribution of dose. The use of three sized aerosols (0.7 μm AMAD, 1.4 μm AMAD, 2.1 μm AMAD) was proposed and exposures to all three sized particles were completed in 1980. Mice from all three exposure groups are being killed at various times during their life to enable the translocation of Pu-239 dioxide from lung to other tissues to be determined. The analysis of these tissues is carried out at AERE.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. G.H. Palmer; Dr. D. Ramsden
Radiological & Safety Div.
Atomic Energy Est.Winfrith
Dorchester
GB-Dorset DT2 8DH

Contract no.: BIO-D-380-81-UK

General subject of the contract:

Plutonium exposures in man : direct monitoring of the lung, re-assessment of the ICRP lung model, and solubility studies.

Description of research work:

Following any accidental intake of plutonium oxide, the assessments of intake, retention and dose commitment are based on a combination of direct lung monitoring, excretion analysis and predictions of the behaviour of the material within the body. The present work is aimed at solving some of the problems in these fields and in increasing the understanding of the variables involved.

The first project continues the studies on the effects of distribution of plutonium oxide within the lung on the multi-detector arrays used for direct monitoring. The second project concludes the re-assessment of the ICRP lung model based on observed data from accidental intakes. The third project is aimed at studying the "in-vivo" solubility of industrial plutonium dusts, studying the variability with time, isotopic composition and irradiation history. The results will be compared with in-vivo data with an aim of being able to make realistic dose commitment calculations at an early stage after any accidental intakes.

The work on the distribution of activity within the lung aims, by means of multi-detector arrays to establish the most likely distribution of activity within the lung for any specific subject. From a knowledge of this distribution the correct calibration factors can be applied and the lung contents calculated. The project will not, per se, lower the present limits of detection which depend on detector type, the isotopic composition of the contaminant, the subjects body build and on predictions of subject background. However the project will remove the main systematic uncertainty in present methods of approach, namely the effects of lung distributions on total body assessment.

The use of a multi-detector/multi-lung region approach requires detailed knowledge of the type and thickness of all intervening chest tissues and a high capacity of on-line and off-line computation facilities.

Planned studies, in this project will cover :

(a) Detectors : Number, type, geometries, responses, backgrounds.
(b) Subject : Chest studies via ultrasonics, X-radiography and encoding of data.
(c) Phantoms : Realism, modelling of lung distributions and tissue distributions.

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(d) Human studies : Controls, inhalation studies, accidental intakes, changes in response with time.
(e) Computation : Matrix approach ; Monte Carlo approach ; perturbation on standard man approach, on-line application.

Observations, both in these laboratories and elsewhere, have shown that the observed lung clearance patterns and the observed excretion patterns following accidental intakes of plutonium are not satisfactorily explained by the present ICRP Lung Model. The project simulates the ICRP lung model and studies the variation in amplitudes and rate constants necessary to predict the observed clearance. It then extends the model to predict urinary excretion behaviour by a semi-empirical approach. The ratio of lung contents/excretion rate for both observed and predicted behaviour is studied as a function of time. Data from project 1 will be included where appropriate. The main data input comes from 15 years of lung monitoring and urine analysis in these laboratories. The project is planned to be concluded in 1981.

Conclusions from projects 1 and 2 will provide much of the information needed to make realistic dose commitment predictions following any accidental intake of plutonium oxide. In order to make assessments early after intake it is necessary to predict the "solubility" of the material within the lung. This will vary with many parameters including particle size spectrum, chemical history of the material and the irradiation history of the fuel. Earlier work at these laboratories has demonstrated with "insoluble" Co-60, Cr-51, Pu-237 that in vitro studies with particles in respirable size range give a soluble fraction and a rate of dissolution that agree with observed lung retention and urinary excretion. It is proposed to extend this work to industrial plutonium dusts with two objectives.

(a) The establishment of a practical technique to augment existing practice in the control of plutonium workers.
(b) Fundamental studies on particle "solubility". In particular does the apparent solubility change with alpha dose to the particles (radiolytic decomposition)? How does this vary with time ? Can induced fission fragment dose be used to extend such studies ? Will the results be compatible with the theory of "ultra-fine" particle transportability ?

This project will be started after completion of project 2.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. J. A. Simmons
Polytechnic of Central London
New Cavendish Street 115
GB-London W1M 8JS

Contract no.: BIO-D-381-81-UK

General subject of the contract:

Microdosimetry of lung.

Description of research work:

There has been considerable controversy in recent years over the potential hazard arising from the deposition of a radioactive particle on or in human tissue. A particular probelm arises when the tissue in question is human lung since it is likely that the radioactive particles may lie, undetected, in a region from which they are not readily removed. The most obvious example is the insoluble material Pu-239 dioxide; particles in the range 0.1 to 1.0 μm diameter may well lie in alveolar sacs for periods which are comparable to, or greater than, the cell turnover time. In such a case the alpha-particles which are emitted may well cause injury to local tissue which could damage the lung as a whole.

The deposition of an alpha-emitting radio-nuclide in the lung can result in a highly inhomogeneous distribution of dose to the organ. However the fundamental weakness of many of the earlier methods of estimating these doses was that the calculations were made for lung tissue of nominal uniform density. This model is not realistic if the ranges of the emitted particles are comparable to the sizes of the inhomogeneities in the tissue. Preliminary measurements and calculations performed under the present contract reveal that effects may be expected at distances of up 750 μm away from the emitter - an order of magnitude greater than previously postulated. It is therefore clear that more measurements need to be made to throw more light on the energies deposited in localized volumes and, furthermore, to establish the results on these groups of cells of such energy depositions.

The method by which specific energy measurements have been made has been described in detail in earlier reports. Briefly, a thin section of rat lung was viewed in an image analyser so that air and tissue were clearly delineated. A dedicated computer noted the positions of each, and an imaginary Pu-239 dioxide particle was considered to have settled in an aveolar sac. The alpha-particles emitted were then followed through the tissue and, using modified energy-loss formulae, their incremental energy losses and total ranges established. Scaling factors involving the use of simple physiological assumptions on sac size and distribution were then used to convert this information to be applicable to human lung.

Now that the technique has been shown to be valid it is being extended so that direct measurements on human lung can be made. It is intended

that more detailed information from several sites in lung should be obtained and it is intended that numbers of cells involved, as well as merely volumes, should be established. Furthermore, in view of the clear evidence that the nucleus is the radiation-sensitive constituent of the cell, it is intended to develop methods of enabling the image analyser to distinguish between the nucleus and the rest of the cell. Thus, eventually, numbers of nuclei which are accessible to alpha irradiation and specific energies deposited in these could be calculated for different lung burdens of radioactive material.
Since the effects of different levels of alpha-irradiation still appear to be a matter of some dispute, attempts will be made to measure these directly. Using appropriate cell-culture techniques, cells of alveolar lung culture would be grown, irradiated and examined for overall viability and the occurrence of chromosome aberrations. Responses would be assessed as a function of dose to cell, dose to nucleus, hits to cell and hits to nucleus in an attempt to establish which is the critical parameter. It should thus be possible to estimate the total volume of alveolar lung (or numbers of cells) at risk.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-D-388-81-UK

Dr. H. Smith
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General subject of the contract:

The transport in and clearance from the body of radionuclides.

Description of research work:

The biological consequences of human exposure to radionuclides is increasingly a cause of public concern. Only by developing our knowledge of the behaviour of radionuclides in the body, of the hazards associated with any exposure and by improving methods of treatment of exposed persons can some of this concern be overcome. Although many radionuclides are potentially of importance plutonium and the higher actinides have probably aroused the greatest concern. Whilst they have been the subject of extensive studies in a number of laboratories in recent years there still remains many areas in which knowledge is very limited. Information is also lacking for some fission products (e.g. Ru-106, Rh-106, Ce-144) and the decay products of radon and thoron.
The investigation of radionuclides may occur during the handling of radioactive materials within nuclear installations and following the release of small amounts into the environment from both nuclear and coal fired power stations.
The objective of the first project is to improve the basis for calculating the dose equivalent to tissues in man following the ingestion of radionuclides. Studies will initially be concentrated on the uptake of actinides, particularly plutonium and americium, but will subsequently be extended to include fission and activation products and other radionuclides for which improved data are required (e.g. Ru-106, Rh-106, Ce-144, Zr-95, Pb-210, Po-210. The programme of work will cover three areas :

(1) Determination of the effect on absorption of factors such as the age and species of the animal, the chemical form, concentration and valence of the radionuclide, incorporation in plant and animal material.
(2) Investigation of the absorption of radionuclides in various species in relation to the uptake of essential ions (e.g. Fe). As the levels of absorption of many essential ions in man are known, this data could provide a basis for extrapolating the results of animal studies on other radionuclides to man.
(3) Estimation of the dose equivalent received by the radiosensitive crypt cells in the gut from activity in the gut lumen and the mucosa.

Previous work has shown that the mechanisms by which the higher actinides plutonium, americium and curium are transported from lungs to

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blood in the early clearance phase following the intake of oxides casts doubt on the validity of currently used metabolic models. It also indicates how in some cases the efficiency of therapeutic regimes can be improved.

In the second project it is intended to extend these studies to define the mechanisms involved in the long term retention and clearance of these oxides which should provide a more rational basis for the assessment of the body content of actinides from bioassay data. Different solubility classes of industrially produced uranium and ruthenium compounds will be included in the programme. The experimental work will involve the identification of the physico-chemical forms of the nuclides in body fluids using a variety of biochemical techniques and examination of their influence on the excretion of these radionuclides.

The third project intends to examine how the efficiency of treatment with the chelating agent diethylenetriamine penta acetic acid (DTPA) can be improved on the basis of knowledge gained from mechanistic studies, viz. either by modifying their reaction with body fluids or by stimulating the formation of transportable species in the lung. It is intended to extend studies with a lipophilic derivative of DTPA, code named Puchel, which suggest that it is more effective for accelerating the removal of actinides from the lungs and body tissued than DTPA alone. Other therapeutic regimes, e.g; macrophage stimulation to remove insoluble materials from the lungs, are at present speculative and will be examined for their potential usefulness.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-D-367-81-D

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Inst. für Genetik
Kernforschungszentrum
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General subject of the contract:

Chelation therapy of actinides : biochemical basis and improvement of treatment procedures.

Description of research work:

The development of safe and effective methods for the removal of actinide elements from the human body following accidental contamination is an important topic in radiation protection research. The methods currently available have only limited effectiveness especially at longer times after exposure or after the intake of insoluble compounds. The development of new or improved methods for the removal of plutonium and related elements from the body based on the use of chelating agents - the so called "decorporation therapy" has formed an important part of the research programme of the Institute for Genetics and for Toxicology of Fissile Materials for many years. The present work on decorporation therapy consists of an integrated programme of fundamental and applied research which has the following basic objectives :

a) The improvement of methods for the removal of transuranium and related elements from the human body following intake of soluble and insoluble compounds by inhalation, through contaminated wounds or by ingestion.
b) The determination of the effectiveness of chelation therapy in reducing the long-term pathological effects which may follow internal contamination by plutonium and related elements.
c) The assessment of the relative risks of chelation therapy, or other types of removal therapy compared to those likely to result from the retention of a significant burden of plutonium-239 or other actinides within the body.

The emphasis in this long-term research programme is directed towards an understanding of the fundamental mechanisms underlying the uptake, transport, cellular deposition and excretion of transuranium elements in mammalian species, the processes underlying the induction of malignant, or other pathological, changes and of the ways in which these mechanisms may be influenced by treatment with chelating agents alone or in combination with other agents.

This research work will be extended by studies in the following areas :

A. Investigation of the biochemical mechanisms involved in transport and tissue deposition of actinide elements after contamination of mammals by soluble or insoluble compounds, with special reference to liver and lung, and of the influence of chelating agents, other types of drug and toxic heavy metals on these processes.

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B. The evaluation of new types of chelating agent based on the modification of substances of known chelating ability by the introduction into the molecule of other chemical groups designed to alter the physiological distribution of the drug or to enhance its effectiveness for the chelation of actinide elements deposited in bone and other tissues. Several such compounds are already available to us and these will be tested for their efficacy in the removal of plutonium and/or thorium following systemic, wound or, later, lung contamination by soluble and insoluble compounds.

These studies, in association with broader studies of the biochemistry of the actinide elements, are expected to lead, through a greater understanding of the underlying biology, to better methods for enhancing the rate of elimination of soluble and insoluble actinide compounds from liver, bone lung and other tissues of contaminated individuals.

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Head(s) of research teams(s):

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Contract no.: BIO-D-377-81-B

General subject of the contract:

Decorporation of heavy alkaline - earth metals and transuranics.

Description of research work:

This project aims to measure the biological response of target cells in bone and bone marrow and to correlate it with the spatial and temporal distribution of dose in the surrounding bone matrix following the deposition of alpha-emitting bone-seekers. Special attention will be paid to Ra-226 and Am-241. If the relation between the behaviour of the target cells and the emitted radioactivity is well defined, it may be possible to predict radiation harm if the local skeletal isotope concentration is known.

Also the effect of decorporative agents on target cells in the critical region will be measured in this study. Up to now great attention has been paid to the amount of toxic substance which is eliminated by the body after decorporative treatment. But, in fact, the therapeutic value of a treatment has to be determined by its eventual success to prevent or reduce radiation effects, and thus by its ability to reduce harm to the target cells. Removal of the isotope may be very small compared to the skeletal burden, but if it occurs from sites which are critical for the induction of radiation effects, then sparing due to treatment may be more important than could be predicted from the overall excretion values.

A. Linking the behaviour of exposed target cells in bone and bone marrow to the amount of energy they absorb from alpha-emitting bone-seekers in their immediate environment requires :

1) information of spatial and temporal responses of radiosensitive cells in bone and bone marrow after contamination with various doses.
2) a detailed knowledge of dose distribution which is non-uniform in time and space.

One typical bone-volume seeker - Ra-226 -, and one typical bone-surface seeker - Am-241 - will be used as radiocontaminants. Two doses of radium, known to induce respectively a low and a high incidence of bone-tumours and two doses of Am-241, which give a comparable amount of energy to the target cells as the two radium doses, will be injected.

Particularly interesting target cells are osteoprogenitor cells which are susceptible to induction of osteogenic tumours, and haemopoietic cells which can give rise to haematological disorders. At different time-intervals after injection, in vivo and in vitro colony assays for haemopoietic cells (respectively the multipotential CFU-s and the

granulocyte-macrophage committed CFU-c) and for osteoprogenitor cell lines (e.g. fibroblast colony forming cells FCFC) will be performed in nucleated cell populations derived from different bone and bone marrow sites. Time-course changes in concentration, absolute number and mitotic activity of the progenitors will be observed. For FCFC also the produced amount of colony stimulating factor can be determined. Measurements of the radioactivity content will be performed in bones corresponding to those where the investigations on the target cells occur and at the corresponding time-intervals. Detailed dose calculations require also profound knowledge of the morphometric characteristics of the selected bone sites which will be consequently investigated.
Cortical (femoral diaphysis) and several trabecular (femoral trabecular end, lumbar vertebrae, sterna) bone sites will be studied separately because the size of their endosteal surface, which is related to the amount of exposed target cells, is different and because of their non-equal isotope labeling.
B. Typical decorporative treatments such as Na-alginate and DTPA, removing respectively heavy alkaline-earths such as Ra-226 and transuraniums such as Am-241 out of the body, will be evaluated for their ability to protect osteogenic and haemopoietic target cells.
1) Direct investigations on CFU-s, CFU-c and FCFC in different bone and bone marrow sites of treated and non-treated contaminated mice will provide information about what happens locally in the critical region and may indicate how treatment may be optimalized.
2) A study on the incidence of tumours, using 800 C57Bl mice, will be continued. The mice are contaminated with three increasing doses of radium, half of the animals are treated with Na-aliginate daily, the others are not treated and are considered as contaminated controls. Two non-contaminated groups are included in the experiment, one treated with Na-alginate and an untreated control group.

In fact, the final effect of any decorporative treatment has indeed to be considered carefully and quite contradictory data exist about this problem. Thus the measurement of the enhanced output of a radiocontaminant is, by itself, not the most reliable method to evaluate the benefit of the decorporating treatment. For example, a study showed a reduction of malignant bone-tumours in mice treated with DTPA after Pu injection, while other results reported in literature showed no protective effect of inhaled Ca-DTPA after Pu inhalation.
The question remains open whether the enhanced removal of alpha-emitting Ra-226 by Na-alginate treatment will reduce radiation effects due to a decreased exposure of the target cells or whether radiation damage will be enhanced due to a continuously increased blood concentration of the isotope.

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Head(s) of research teams(s):

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Contract no.: BIO-D-374-81-NL

General subject of the contract:

Carcinogenicity of iodine-123, -125 and -131 in the mouse.

Description of research work:

Differences in radiation damage of the thyroid will occur after application with various iodine isotopes. The damage will mostly be determined by the dose distribution within the thyroid dependent on the isotope used and to a lesser extent by the dose rate. Heterogeneity of radiation doses within the thyroid will depend on the energy, and thus the path-lengths, of the beta-particles. Furthermore, the effective half-life in the thyroid will determine whether a certain dose-commitment will be given in a short or in an extended period.

In order to evaluate the importance of these factors in the thyroid, scientist at the Radiobiological Institute TNO determined the radiation damage of the thyroid by decrease in thyroid function after injection with various doses of three iodine isotopes in young mice. The three isotopes are : 1) I-123, which is an isotope with a very short physical half-life (0.55 days) and an average beta energy comparable to I-13; 2) I-131, which has a half-life of 8 days. 3) I-125, which has a longer half-life (60 days), and its radiation dose in the thyroid is partly due to very low energetic electrons and beta particles.

In order to be able to correlate the reduction in thyroid function with radiation dose at the relevant site, calculations were made of the radiation dose in the follicle cells of the thyroid by Monte Carlo procedures. From the obtained dose-effect curves, a reduction in tracer uptake to 20 per cent of the value for untreated mice was calculated to occur in mice injected with 35 MBq of I-123, 13 MBq of I-125 or 2.2. MBq of I-131. The corresponding calculated average dose commitment to the follicle cells amounted to 110 Gy, 440 Gy and 380 Gy, respectively. This relative effectiveness of these isotopes is in good agreement with the dose rate of exposure. If, in contrast, average dose to the whole thyroid gland is used, the relevant values are 170, 1700 and 340 Gy indicating that indeed a much better fit is obtained between biological effect and dose if the dose estimates are made at the level of the follicle cells.

The Radiobiological Institute TNO now proposes to study the tumour induction in the thyroid gland after a similar scheme of injected doses of the three isotopes as was used for the thyroid function studies. In to adjust the calculated radiation doses for differences in experimental conditions, such as iodine uptake and retention, these parameters will again be determined in new experiments. The Radiobiological Institute TNO will use the results of a pilot study on

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the carcinogenicty of these isotopes as a guide to determine : a) the required number of mice per dose group; b) whether in case of a very low tumour incidence, treatment with thio-uracil must be used to increase this frequency; c) the time after isotope injection at which surviving mice have to be killed to determine the tumour induction (1 1/2 - 2 1/2 years).

On the basis of this information the Radiobiological Institute TNO will perform an experiment to study the thyroid tumour frequency in mice after different doses of I-125 and I-131. If conditions permit, studies on iodine 123 will also be included. The mice killed at the end of the experiment and those found or killed when moribund will be subjected to a gross necropsy. The thyroids will be sectioned semiserially, stained and examined by light microscope. From the information obtained in this study the relation between radiation dose, decrease in thyroid function and carcinogenicity of the three isotopes can be determined.

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Head(s) of research teams(s):
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Contract no.: BIO-D-384-81-UK

General subject of the contract:

Distribution and long-term retention of insoluble radioactive particles in the lungs of man and rat.

Description of research work:

The study of the long-term retention of insoluble particles in the lungs of man aims at identifying areas in the lung in which radioactive material may be retained for a number of years and subsequently to calculate the risk to adjacent target cells. Lungs will be obtained at post-mortem from people known to have ceased exposure to an identifiable insoluble non-radioactive material 1 to 40 years previously. The subjects will be for example workers retired from a light industry involving exposure to an identifiable insoluble particulate. Tissue will be taken from sites representing all anatomical features of the lungs, trachea and associated lymph nodes. For each site the tissue will be examined at the microscopic level to measure (a) content of naturally occurring U-235 and (b) content of material to which the subject had ceased exposure.

The U-235 will be used to indicate possible sites of long-term retention and the non-radioactive particulate will provide information on the period of retention of particulates.

The microscopic site of U-235 will be identified by a combination of neutron-induced autoradiography and alpha-imaging. From these same sections using the Quantimet 720 and a modification of the computer programme, calculations will be made of the likely alpha-particle dose to the bronchial epithelium and other target tissues.

The study of the long-term retention of insoluble particles in the lungs of rats aims at : (a) establishing the route(s) of clearance of insoluble radioactive particles deposited in the alveolus and (b) determining those microscopic sites within the lung and its lymphatic drainage system in which particulate matter is retained for long periods (sites of long-term retention). To this extent $^{239}PuO_2$ and other particles of various sizes will be deposited in the lungs of adult rats. Particles will be implanted directly into the alveoli either by microsurgical technique on exposed lung and/or by other means. Animals will be killed at intervals of up to 2 years and serial sections are used to establish the pathways of clearance and sites of long-term retention. It is intended to examine the tissues and particle distribution in them by light microscopy, electron microscopy and using neutron induced autoradiography. Tissues prepared for the latter technique will be examined with an image analysis system (Quantimet QTM

720 and PDP 11) to determine the particle distribution in relation to particular cells with the aim of calculating absorbed dose to specific target cells in the lung. The results from this study will be compared closely with those from the study on man in order to establish the value of the rat as a model for man in studies of long-term insoluble particle retention. Such information could be useful in subsequent attempts to investigate ways of reducing the probability of insoluble radioactive particles reaching or remaining in the long-term retention sites.

The interaction in vitro of human, and rat lung macrophages with insoluble actinide paticles will be evaluated to establish some of the biochemical and physical factors that govern the uptake and retention of insoluble radioactive particles by lung macrophages.

Insoluble particles, including those of $^{239}PuO_2$ used in the study on rats, will be used. The human macrophages will be obtained mainly from surgical lobectomy specimens and in the rat by lavage of excised lungs. Initial work will attempt a classification of the lavaged lung macrophage population based on morphology or function. Subsequently the sub-populations of macrophages will be examined for their response to the different types of insoluble particle. This in vitro research will be used to explain aspects of the behaviour of the lung macrophages seen in vivo during the study of rats.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-D-385-81-UK

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General subject of the contract:

Studies in radiation carcinogenesis and the interaction of radiation and chemical carcinogens.

Description of research work:

1. Study of radiation induced skin tumours in mice. The laboratory has, for many years, undertaken experiments in which mice are irradiated with an external source of beta-particles so that about 20 cm2 of skin is exposed without underlying tissues or organs being affected and consequently immunological damage is avoided. Earlier work using a small range of single doses and fractionated doses has already been published. A study of variations in incidence of skin tumours using 4 different dose-rates is nearing completion and these data will also provide more detailed information for dose-response curves. A recently completed study using multiple fractionations indicated the need for dividing doses into 2 unequal fractions and relating tumour incidence to early clinical effects on the skin. Some observations have been made and these shall be extended. This localized skin irradiation shall be combined with a range of doses of whole-body penetrating X-irradiation, some of which will be large enough to interfere with the immune competenc of the mice. A further extension will include observations on the interaction of beta-irradiation with other physical, and also perhaps chemical agents, on the incidence of skin tumours.
2. Study of the effects of radiation en ENU induced tumours in rats. Ante-natal irradiation increases the incidence of nervous tissue tumours in children. This has not been confirmed in experimental animals but, on the other hand, experiments have been reported in which the incidence of the central nervous tissue tumours which follow the exposure of fetal rats to the carcinogen ethylnitrosourea (ENU) was reduced by irradiation. This effect, in which radiation appears to be reducing tumour incidence, might have been due to radiation effects on the mother or placenta reducing the dose of ENU to the fetus. It has been found that the laboratory's HML strain of inbred rats produced nervous tissue tumours when given ENU shortly after birth and it is propose to follow the interaction of X-irradiation and ENU given within 48h afther birth. Initially the absorbed doses will be 20 rad and 125 rad so if tumour incidence is reduced there will be some indication of the radiosensitivity of the cells concerned. As well as some rats being given ENU alone others will be given irradiation alone. Recent work in this laboratory has shown that mice, irradiated as young adults, develop myeloid leukaemia only after relatively low doses

(75-300 rad). Thus as the rats in this project will be irradiated when their development is still incomplete (as is the human fetus during ante-natal irradiation), they will provide an opportunity of finding whether the induction of nervous tissue tumours, like myeloid leukaemia, occurs only after low doses. According to the outcome of the first phase of the experiment, variations will be made in the doses of ENU and of X-rays, and the order in which they are given.

3. Radiation carcinogenesis in rabbits. Because of their longevity it is very unusual for rabbits to be used in an experiment in which they are allowed to live out their natural life-span. Even after high doses a rabbit irradiated at an age of 9-12 months can live a further 7-9 years. In spite of this the late effects in rabbits given whole-body irradiation of either fission neutrons or gamma-rays have been studied. The incidences of osteosarcomas and fibrosarcomas were exceptionally high. The usually very rare Sertoli-cell tumour of the testis was significantly increased in numbers and basal-cell tumours in the skin, also very rare in rabbits, occured in large numbers. The present work is concerned with whole-body gamma-irradiation at 3 different dose-rates, using absorbed doses of 100-1100 rad. Many animals, but by no means all, needed for the experiment have been irradiated and all will have to be observed for many years. Further extension of the work will probably be along the lines of half-body irradiation afther high doses, to preserve immunological function, and of irradiationg younger rabbits, in particular to note any difference in the types of tumours encountered.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-D-390-81-D

General subject of the contract:

Late somatic effects of ionizing radiation in the mammalian organism.

Description of research work:

The contract will foster cooperation between research laboratories in Europe engaged in work of importance on late biological effects of ionizing radiation. Emphasis is given to : a. the definition of dose and time parameters and of other environmental hazards resulting in late effects of radiation. b. the elucidation of etiological mechanisms of late effects with the view of modifying the chain of events leading to late damage and thus preventing such damage. c. the development of early indicators of late damage so that preventive or restorative measures can be taken as soon as such damage becomes detectable. d. the search for epidemiological and experimental models which allow a better estimate of potential risks to man. The scientific work is carried out by committees dealing with the standardisation of procedures and the coordination of research programs. Although the program proposed flows directly from ongoing research projects, it also shows some shift in emphasis to epidemiology, to measure directly the frequency and severity of radiation induced late effects, to the effects of low doses and to late effects of radiation on the developing organism. The new program also envisages a closer cooperation with certain laboratories outside Europe (U.S.) equipped with highly specialized facilities to carry out studies which could otherwise not be performed by the means available in Europe. 1. Two of the working committees are concerned with the establishment and maintenance of high standards of scientific work i.e. on dosimetry and on pathology. a) The Committee on Dosimetry has achieved high level of dosimetric accuracy by regular intercomparisons, in site checks and education. This will continue in the future, particularly for not yet standardized conditions of partial body exposure, for neutron irradiation and for exposure by internal emitters. b) The work of the Pathology Committee will continue along the established lines. Thus, the EULEP Pathology Atlas will be completed, seminars and information will be given on the increasingly greater variety of histological and cellular techniques and methods of quantitative morphology available for the study of late effects of radiation. 2. The research committees coordinate the scientific work of the member laboratories dealing respectively with non-neoplastic late effects, neo-plastic diseases and the effects and dosimetry of internal emitters.

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Limits to occupational and public exposure must, for certain tissues, be based not on the risk of cancer induction but on the so-called non-stochastic effects. These are non-neoplastic slowly developing but disabling forms of tissue damage which have always limited radiotheray of cancer. Such effects may have particularly serious consequences after irradiation of the developing organism. Common denominators of these effects are vascular alterations and fibrosis. The programme on vascular alterations attempts to trace the link between early modifications in the vascular system of the brain and the subsequent late lesions and will correlate these to a variety of morphological, biochemical and functional endpoints. Attention is also focused on changes in structure and number of glia cells. Two groups plan to extend these studies to the developing organism. Another project on vascular alterations utilizing the hamster cheek pouch, a relatively simple model studies the relation between cellular and vascular alterations. Structure and cellular knetics of the vascular and associated nervous system will be investigated in dependence on dose and time after irradiation. The study of the mechanism of fibrotic changes will continue to concentrate on lung in view of the importance of lung fibrosis for human pathology. The relationship between the development of late fibrosis and early changes in permeability and blood clotting as well as specific functions related to the metabolism of biogenic amines and hormones will be studied. Cancer induction is a very wide field and a considerable range of tumor types is being studied in the various EULEP laboratories. The experimental aims are to define the cells from which neoplasm originate, the conditions of radiation and other environmental factors, virus, chemical carcinogens, which result in cancer induction and the possibilities to extrapolate experimental data from animals to man. One project studies the behaviour of cells prior induction of leukemia using a chromosomal marker developed in an EULEP laboratory to distinguish between donor and host cells.

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Head(s) of research teams(s):

Contract no.: BIO-E-404-81-NL

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Dept. Cell Biology
and Genetics
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NL-3000 DR Rotterdam

General subject of the contract:

The genetic and biochemical basis of radiation sensitivity in human and other cells in culture.

Description of research work:

The purpose of the project on the genetic analysis of DNA repair is the identification of genes involved in the repair of DNA damage in eukaryotic cells, in particular those of human origin. The laboratory will contribute to this project through the :

a. collection of cell strains from patients with radiation sensitive syndromes and isolation of repair deficient mutants from cultured mammalian cells.
b. characterization of repair mutants using the techniques of somatic cell genetics (hybridization, cybridization and gene transfer).
c. identification of repair genes using recombinant DNA technology.

Cell strains will be established from patients suffering from sensitivity to DNA damaging agents (xeroderma pigmentosum, XP, ataxia telangiectasia, AT, Fanconi's anemia, FA and other genetic diseases in which defective repair of DNA damage may play a role). With regard to the isolation of repair mutants from cultured cells we will apply a new technique for replica plating of clones of cultured cells. We will also try to isolate mutants from cell lines which are able to differentiate in vitro (teratocarcinoma cell lines).

During the past 8 years our laboratory has been involved in a complementation analysis by cell fusion, which has facilitated a genetic analysis of repair defects in XP cells. These studies will be continued. So far this analysis has been performed on hybrid bi- or multinucleated cells, which do not proliferate. By isolation and culturing of proliferating mononucleated hybrid cells we will try to investigate complementation and the biological consequences of it in these proliferating hybrids. These cells have the advantage that genomic interaction can take place inside one nucleus. Moreover, repair test systems can be used which require large numbers of cells and/or cell proliferation (e.g. post-replication repair).

The interaction of the nucleus and the cytoplasm in complementation will be investigated by fusion of cytoplasts of one repair mutant with complete cells of another mutant and vice-versa (cybridization).

Recently techniques have been developed which provide the introduction of foreign genes into mammalian cells. These techniques are based on the selection of cells which show a high incidence of uptake and incorporation of foreign DNA in their genome. These methods will be applied in the genetic transformation of repair deficient mammalian cells into repair proficient cells. Isolation and molecular cloning of

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the transforming fragments using recombinant DNA technology may facilitate the identification of human DNA repair genes. These studies will be carried out in collaboration with Dr. P.H.M. Lohman and Dr. P.H. Pouwels (Contract BIO-E-403-81-NL), Dr. A.L. van der Eb, University of Leiden (Contract BIO-E-405-81-NL) and Dr.P.van de Putte, University of Leiden (Contract BIO-E-407-81-NL).

In the project on biochemical analysis of DNA repair, our studies on the biochemical nature of the repair defects in mutants cell lines established in the first project will be continued. In collaboration with Dr. Lohman and coworkers new techniques will be introduced in these studies.

In the project on the consequences of DNA damage and repair our efforts will concentrate on the study of possible relationships between DNA repair, cell differentiation and carcinogenesis. The pleiotropic clinical effects of repair deficiencies in man suggest a role of DNA repair mechanisms in cell differentiation and in transformation into tumour cells. The culture of teratocarcinoma cells provides a system for the study of cell differentiation in vitro and in vivo. Two different approaches are possible: a study of the influence of cell differentiation on the repair capacity of the cells and an analysis of the effects of DNA damaging agents on differentiation capacity of the cells. When we are able to isolate repair deficient mutants of teratocarcinoma cells (see first project) the differentiation characteristics of these cells after treatment with DNA damaging agents will be investigated in vitro and in vivo (in tumours and, if possible, in chimaeric mice). With regard to carcinogenesis we will investigate cell lines obtained from tumours of patients having a repair deficiency (predominantly xeroderma pigmentosum).

These studies will include cytogenetic characterization of the tumour cells and analysis of the malignant characteristics of the cells by hybridization.

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Head(s) of research teams(s):

Prof. B.A. Bridges
Cell Mutation Unit, MRC
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Contract no.: BIO-E-414-81-UK

General subject of the contract:

The genetic and biochemical basis of radiation sensitivity in human and other cells in culture.

Description of research work:

Work from this laboratory on Project 1 will consist of the collection of cell strains from patients with radiation-sensitive syndromes, from indivuduals heterozygous for these disorders, and from individuals with certain neurological disorders. These will be used in the studies detailed in Project 3. Work from this and other laboratories over the past five years has identified a large number of human variants with increased sensitivity to radiation and chemical mutagens. The biochemical characterization of the presumed defect in DNA repair in these variants using conventional techniques (e.g. excision of damage, repair replication, repair of strand breaks) has met with less success. Preliminary work with the UV-sensitive Cockayne syndrome cells has provided some encouraging results. It has been discovered that the kinetics of DNA replication after irradiation are abnormal in Cockayne cells. One possible interpretation of the data is that Cockayne cells fail to synthesize a protein responsible for the restoration of normal rates of DNA synthesis in irradiated cells. If this interpretation is confirmed it is proposed in project 2 to try to identify new proteins synthesized after irradiation. If such proteins can be detected the human variants could then be screened for the presence of these proteins. Recently a number of new enzymes (DNA glycosylase) each of which acts specifically on a particular damaged base in DNA, have been identified principally in bacteria. There is evidence that these enzymes participate in DNA repair and it is likely that enzymes of this type are involved in the repair of damage produced by ionizing radiation. It is proposed to look for such enzymes in human fibroblasts with a view to comparing normal and repair-deficient cells. A new type of enzyme activity has been shown to be induced by treatment of bacteria with low doses of alkylating agents. This activity increases the resistance and decreases the mutability of the bacteria when exposed to a challenging dose of the alkylating agent. This phenomenon, termed adaptation, will be sought in mammalian cells using both enzymatic and cellular approaches, and its involvement in the response to ionizing radiation will be investigated. Finally the acute changes in NAD metabolism which occur in response to ionizing radiation and alkylating agents, and the recently discovered involvement of poly ADP-ribose polymerase in recovery from DNA damage, will be investigated in normal and abnormal cells. Mutation to 6-thioguanine resistance can be monitored efficiently in cultured human fibroblasts using the

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so-called roller bottle technique. It is proposed in project 3 to continue to use this technique to describe both spontaneous and induced mutation in the array of repair-deficient cell strains now available. Experience with mutation induction in rodent cells has shown that cells may respond specifically to DNA damage with respect to the classes of mutants expressed. It has become necessary, therefore, to extend the range of mutational end points which are assayed in human cells. Some developmental work on the use of ouabain resistance has already been completed and it is intended to evaluate alternative selective systems such as resistance to diphtheria toxin. Cellular sensitivity at the level of lethality together with increased cancer proneness has already been observed in ataxia telangiectasia heterozygotes. A study of mutation in heterozygotes of this and other repair-defective syndromes such as Fanconi's anaemia is proposed. What little is known of mutation in the human repair-defective syndromes shows that the X-ray sensitive ataxia telangiectasia cells are different from the UV-sensitive xeroderma pigmentosum cells in showing no correlation between hypersensitivity and hypermutability suggesting that the mechanisms for sensitivity and mutation can be separated in human cells. It is proposed to examine this area with other X-ray-sensitive strains and with other mutation selective systems which are under development to establish the generality of this effect. In addition to the cellular sensitivity to specific DNA damaging agents, neurological defects are a common feature of many of the human repair-defective diseases. An attempt will be made to confirm this correlation by a study of diseases such as Huntigton's chorea where neurological stigmata are the prime feature. Although it is becoming increasingly clear that radiation and many chemicals cause cancer by damaging DNA, the somatic mutation theory of cancer is far from proven. A careful study of the events that occur after a mutation becomes fixed in the DNA should help to provide evidence either for or against somatic mutation, and may well assist in the evaluating of carcinogenic risks of radiation. In order to relate somatic mutation to carcinogenesis, it is necessary to account for the very long latent period between the initial DNA damage and the final appearance of a tumour. For this reason, it is proposed to characterize the steps involved in the expression of induced mutations and to determine any effect of tumour promoting agents such as phorbol esters on the process. Initially, mouse lymphoma L5178Y cells are likely to be most suitable for the type of approach we envisage. As technology improves, human cells may also be used. Photoreactivation has proved to be a very useful tool in the investigation of mechanisms of microbial mutagenesis. It is proposed to utilize the ability of marsupial cell lines to perform photoreactivation in studies of lethality, chromosome damage and mutation.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. J.E. Celis
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DK-8000 Aarhus C

Contract no.: BIO-E-416-81-DK

General subject of the contract:

Changes in gene expression resulting from primary mutational events accompanying X-ray irradiation induced transformation in human cells.

Description of research work:

It will be attempted to unravel the changes in genetic expression resulting from primary mutational events accompanying X-ray irradiation induced transformation in human cells. The following lines of experimentation will be carried out :

1. Development of an assay to distinguish between normal and cancer cells in culture. It is hypothesized that cell function and behaviour can be altered through partial or total inactivation of genes which regulate cell proliferation and that gene inactivation is achieved mainly by mutation (irradiation or chemically induced). It is then reasonable to assume that normal and cancer cells will differ in a few polypeptides whose rate of synthesis (increase, decrease, appearance of new polypeptides in cancer cells) could be related to cell growth and/or to the maintenance of the malignant state. Therefore, a detailed comparative study using high resolution two dimensional gel electrophoresis of the polypeptides synthesized by a large number of human normal/tumor cell pairs as well as the analysis of polypeptide changes that take place during normal processes (cell cycle, ageing of primary human fibroblasts) should reveal changes which govern or result directly from the principal event of the transformation and or the malignant process. Since in many cases it is difficult to determine the exact origin of the tumour cells, it will be necessary to carry out an extensive study of the polypeptide synthesized by tumour cells irradiated with different doses of X-rays.
2. Preparation of antibodies against polypeptide(s) markers for transformation and or tumorigenicity. Once the polypeptide(s) have been identified and purified by two dimensional gel electrophoresis, they will be used to prepare monoclonal antibodies. Emphasis will be given to polypeptide markers that are mainly present or that are irreversibly repressed in transformed cells.
3. Changes in gene expression accompanying the irradiation induced transformation process. The synthesis of transformation sensitive polypeptides in human cells irradiated with different doses of X-ray will be determined with the monoclonal antibodies using the double immunofluorescence technique. Under these conditions it is possible to detect changes in a single cell among a large population of irradiated cells.
4. Microinjection of marker polypeptides into normal, tumour and X-ray irradiated cells. Effect on the pattern of polypeptide synthesized by

the injected cells. Since marker polypeptides are expected to regulate the synthesis of various other proteins, their microinjection into normal or transformed cells followed by (^{35}S)-methionine labelling and two dimensional gel electrophoresis should produce an in vivo assay for their activity.

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Head(s) of research teams(s):

Contract no.: BIO-E-419-81-F

Dr. M. Dalebroux
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General subject of the contract:

Study of irradiation effects on genetic markers.

Description of research work:

In project one a study on the nature of radio-induced genetic events in the a_1-a_2 system of tobacco and study of their relative frequencies in terms of varying doses of acute and chronic irradiation is carried out and a search is made for repair-deficient mutants. Genes a_1 and a_2, obtained by mutagenic treatment and localized on chromosomes R and S, respectively, of Nicotiana tabacum L., determine, according to their dosages and associations with their wild alleles, a_1^+ and a_2^+, different colors of the palisade tissue. On genotype a_1^+/a_1 a_2^+/a_2 greenish yellow in color and with a chlorophyll content about half of that of genotype a_1^+/a_1^+ a_2^+/a_2^+, discontinuous variations of different types were observed. By means of in vitro isolation and regeneration into full plants of cells from "mutated" areas, it was shown that all variations involve genetic information. The genetic changes can be attributed to deletions, conversions, translocations and somatic crossing-overs. The varying tissue thus constitutes a cell clone that marks the ontogenic lineage of shoot meristems, and may be regarded as an excellent system for investigating at the cellular level the mutagenic effects of radiations, acute or chronic, and, eventually, of chemical pollutants. The different alleles available are : at locus a_1: a_1^+, wild allele; a_1, strongly antimorphic to a_1^+ and a_2^+; a_1^0, amorphous allele or small deletion : at locus a_2 . a_2^+, wild allele; a_2^{yg}(yg), slightly antimorphic to a_1^+ and a_2^+; $a_2^0(a_2)$, amorphous allele or small deletion. It is possible to obtain a genotypic series involving the different alleles mentioned above. By comparing different genotypes with respect to their reactions to irradiation, it is possible to evaluate the relative importance of genetic events due to somatic crossing-over, conversion, mutation, deletion and translocation. For example, two homozygous genotypes, a_1^+/a_1 a_2^+/a_2^+ and a_1^+/a_1^+ yg/yg, rather rarely yield twinned variations spots. These variations are due to events other than somatic crossing-over. However, these phenotypes, which can result from translocations between homologous, homoeologous or different chromosomes cannot be distinguished from those arising from nondisjunction. It is proposed to quantify the relative frequencies of the different types of genetic events in terms of varying doses of irradiation. This research will be carried out under two different general conditions : (1) under acute gamma irradiation, and (2) under conditions of chronic irradiation given by rocks taken from a number of uranous outcrops from south-west France. It is also proposed to carry

out a program consisting of the search for repair-deficient mutants in Nicotiana tabacum var. Xanthi n.c.. Attempts will be made to introduce such markers in genotypes involving different combinations of alleles of the a_1-a_2 system so that the impact of repair ability on the relative importance of the radio-induced genetic events could be observed and assessed. The research would be performed by the method of replicas on colonies of mutagenized haploid protoplasts. Under the assumptions (1) that forty independent repair loci are involved and (2) that the mutation probability under mutagenic treatment of any one of these loci is equal to 10^{-4}, the observation of a sample of 750 colonies will yield at least one gamma-sensitive mutant with a probability larger than 0.95.

In project two a study is made of genetic effects of low and very low, external and internal, chronic irradiations (natural and artificial) as well as a comparison and interactions with other environmental factors. On the basis of available results, it is proposed to study, in the laboratory, gentic effects of low and very low, both external and internal, chronic doses of radiations. The research will be carried out by means of natural radionuclides (U-238, U-233) as well as the artificial radionuclide H-3, which is one of the most abundant nuclear energy tailings. The genetic markers will be (1) the a_1^+/a_1 a_2^+/a_2 system of Tobacco, and (2) the waxy locus in Barley pollen. For the Tobacco system as well as for that of Barley, the experimental unit is the CELL. This means that very large populations can be handled to make reliable statistical conclusions drawn on relatively small observed variations. The expected results will help evaluate, on experimental bases, the margins for safety in order to confirm or revise the present norms in radioprotection. From a more general point of view, the results would allow (1) to anticipate the eventual genetic impact of nuclear activities and (2) to contribute to the assessment of the eventual detriment due to radiations. In case genetic impact and detriment are large enough to be evaluated, it is proposed to use the same genetic markers to evaluate the effects of other environmental factors, natural or artificial, related to activities which do not involve nuclear energy. To do this, the markers would be observed in various environmental situations (rural, urban, industrial); as a matter of fact, it would be unwise, or even dangerous, not to make this comparison, as it is imperative to "situate" the effects of low and very low doses of ionizing radiations with respect to those of known factors interfering in the daily life. It is also important to keep in mind that, most of the time, synergy is the rule. Therefore it is necessary to study the interactions of various factors. However, these factors are so numerous that a choice must be made. Given the present concern and knowledge, it is proposed to start a first set of experiments to study the interactions between ionizing radiations and electrophilic compounds, substances that have been shown, or are suspected, to be carcinogenetic.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-E-402-81-NL

General subject of the contract:

The exploration of methods for evaluating the induction of autosomal meiotic non-disjunction in the mouse.

Description of research work:

The research program is a continuation of a study on chromosome behaviour during male and female meiosis in the mouse. The proposed research explores chromosome mutants to test the inducibility of non-disjunction by ionizing irradiation (and potential chemical mutagens). The knowledge of the origin of spontaneous chromosome misbehaviour during meiosis in these chromosome mutants can be used for understanding deviant chromosome behaviour after treatment with ionizing irradiation or chemical mutagens. For measuring induced responses, chromosome mutants are chosen with known and high spontaneous meiotic non-disjunction rates, postulating that such systems are more sensitive to a changing cellular environment than normal systems. Since a control level of well defined non-disjunction is available, a large range from negative to positive induced effects can readily be analysed in a strictly quantitative manner. The use of heterochromatin polymorphisms is included. These studies have yielded and will yield new information about the origin of spontaneous and induced non-disjunction during meiosis. The programme is centered round anaphase I originating non-disjunction. By analysing the chromosome complement (total number of chromosomes and presence of markers) in the second meiotic division cells, the effects can be measured quantitatively in both sexes. The meiotic stages during which the irradiation will be applied are a) zygotene/beginning of pachytene as the meiotic pairing phase (the integrity of the synaptonemal complex can be monitored by electron microscope whole mount spreading techniques) and b) end diakinesis/metaphase I as important stages for co-orientation and segregation of the chromosomes. A first study (under contract n° 403-78-3 ECI N/MB) has already yielded : information concerning the duration of the period median metaphase I - median metaphase II (less than 3 hours) and the effects of a low dose of fission neutrons and X-rays on the level of anaphase I - originating non-disjunction in Rb (11.13)4Bnr/+ male mice. In the experiments proposed, low doses (< 15 rad) and low dose rates (< 1 rad/min) of fast neutrons (mean energie 1 MeV) will be used again. For comparison, X-rays are included. Recently a technique has been developed to obtain specific cell stages of spermatogenesis. The true induction effects as measured at metaphase II, not influenced by (partially) incorrect timing or cell delay due to the treatment of cells which were already post anaphase I at the moment of irradiation, can be obtained using

this technique. The following four sets of material are considered promising for evaluating induced meiotic non-disjunction in male and female meiosis :
1) Heterozygotes for the Rb(11.13)4Bnr Robertsonian translocation, characterized by about 20 % anaphase I non-disjunction in the male and an even higher percentage in the female.
2) Heterozygotes for the T(1;13)70H reciprocal translocation which display in the male "4-9% numerical non-disjunction" and 25 % adjacent-2 segregation (homologous centromeres move to the same pole) at anaphase I. Concerning the latter percentage, evidence has been presented for an influence of paracentromeric chiasmata in the longest interstitial segment of the pairing cross at diakinesis/metaphase I on adjacent-2 segregation.
3a) Double heterozygotes for two translocations both involving chromosomes 1 and 13, but with slightly deviant breakpoint locations. This will lead to a region of non-homology in the two bivalents, formed by the four translocation chromosomes, probably enhancing the level of the small bivalent-caused univalents.
3b) Double heterozygotes for two reciprocal translocations both involving chromosomes 2 and 8, but again with slightly deviant breakpoint positions; the implications being the same as in the foregoing construction.
4a) Homozygotes for the T(1;13)70H reciprocal translocation. In females ageing appears to increase the level of 1^{13} small marker bivalent-caused univalence during diakinesis/metaphase I. The consequence is a proportional increase of aneuploid secondary oocytes for this chromosome.
4b) Homozygotes for the T(1;11.13 S)70H reciprocal translocation. This translocation is a combination of the T(1;13)70H originating long marker with the metacentric Rb(11.13)4Bnr marker forming a very long new submetacentric chromosome in the presence of the small, T70H originating, 1^{13} marker. The first breeding results suggest an increased level of spontaneous non-disjunction. Further research is indicated.
All chromosomal rearrangements are on a Swiss random bred genetic background. Isolated in it too, is a chromosome 13 originating from Mus musculus molossinus (Japanese house mouse), which in comparison with the "homologous" Swiss chromosome, contains very little heterochromatin. So the role of heterochromatin in non-disjunction can be studied in various combinations (homozygous/heterozygous and combined with the chromosome mutants under points 1-4).

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. M. Delpoux
Service de Botanique
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Allées J. Guesde 39
F-31077 Toulouse Cédex

Contract no.: BIO-E-430-81-F

General subject of the contract:

Study of irradiation effects on genetic markers.

Description of research work:

In project one a study on the nature of radio-induced genetic events in the a_1-a_2 system of tobacco and study of their relative frequencies in terms of varying doses of acute and chronic irradiation is carried out and a search is made for repair-deficient mutants. Genes a_1 and a_2, obtained by mutagenic treatment and localized on chromosomes R and S, respectively, of Nicotiana tabacum L., determine, according to their dosages and associations with their wild alleles, a_1^+ and a_2^+, different colors of the palisade tissue. On genotype $a_1^+/a_1\ a_2^+/a_2$ greenish yellow in color and with a chlorophyll content about half of that of genotype $a_1^+/a_1^+\ a_2^+/a_2^+$, discontinuous variations of different types were observed. By means of in vitro isolation and regeneration into full plants of cells from "mutated" areas, it was shown that all variations involve genetic information. The genetic changes can be attributed to deletions, conversions, translocations and somatic crossing-overs. The varying tissue thus constitutes a cell clone that marks the ontogenic lineage of shoot meristems, and may be regarded as an excellent system for investigating at the cellular level the mutagenic effects of radiations, acute or chronic, and, eventually, of chemical pollutants. The different alleles available are : at locus a_1: a_1^+, wild allele; a_1, strongly antimorphic to a_1^+ and a_2^+; a_1^0, amorphous allele or small deletion : at locus a_2 : a_2^+, wild allele; a_2^{yg}(yg), slightly antimorphic to a_1^+ and a_2^+; $a_2^0(a_2)$, amorphous allele or small deletion. It is possible to obtain a genotypic series involving the different alleles mentioned above. By comparing different genotypes with respect to their reactions to irradiation, it is possible to evaluate the relative importance of genetic events due to somatic crossing-over, conversion, mutation, deletion and translocation. For example, two homozygous genotypes, $a_1^+/a_1\ a_2^+/a_2$ and a_1^+/a_1^+ yg/yg, rather rarely yield twinned variations spots. These variations are due to events other than somatic crossing-over. However, these phenotypes, which can result from translocations between homologous, homoeologous or different chromosomes cannot be distinguished from those arising from nondisjunction. It is proposed to quantify the relative frequencies of the different types of genetic events in terms of varying doses of irradiation. This research will be carried out under two different general conditions : (1) under acute gamma irradiation, and (2) under conditions of chronic irradiation given by rocks taken from a number of uranous outcrops from south-west France. It is also proposed to carry

out a program consisting of the search for repair-deficient mutants in Nicotiana tabacum var. Xanthi n.c.. Attempts will be made to introduce such markers in genotypes involving different combinations of alleles of the a_1-a_2 system so that the impact of repair ability on the relative importance of the radio-induced genetic events could be observed and assessed. The research would be performed by the method of replicas on colonies of mutagenized haploid protoplasts. Under the assumptions (1) that forty independent repair loci are involved and (2) that the mutation probability under mutagenic treatment of any one of these loci is equal to 10^{-4}, the observation of a sample of 750 colonies will yield at least one gamma-sensitive mutant with a probability larger than 0.95.

In project two a study is made of genetic effects of low and very low, external and internal, chronic irradiations (natural and artificial) as well as a comparison and interactions with other environmental factors. On the basis of available results, it is proposed to study, in the laboratory, gentic effects of low and very low, both external and internal, chronic doses of radiations. The research will be carried out by means of natural radionuclides (U-238, U-233) as well as the artificial radionuclide H-3, which is one of the most abundant nuclear energy tailings. The genetic markers will be (1) the a_1^+/a_1 a_2^+/a_2 system of Tobacco, and (2) the waxy locus in Barley pollen. For the Tobacco system as well as for that of Barley, the experimental unit is the CELL. This means that very large populations can be handled to make reliable statistical conclusions drawn on relatively small observed variations. The expected results will help evaluate, on experimental bases, the margins for safety in order to confirm or revise the present norms in radioprotection. From a more general point of view, the results would allow (1) to anticipate the eventual genetic impact of nuclear activities and (2) to contribute to the assessment of the eventual detriment due to radiations. In case genetic impact and detriment are large enough to be evaluated, it is proposed to use the same genetic markers to evaluate the effects of other environmental factors, natural or artificial, related to activities which do not involve nuclear energy. To do this, the markers would be observed in various environmental situations (rural, urban, industrial); as a matter of fact, it would be unwise, or even dangerous, not to make this comparison, as it is imperative to "situate" the effects of low and very low doses of ionizing radiations with respect to those of known factors interfering in the daily life. It is also important to keep in mind that, most of the time, synergy is the rule. Therefore it is necessary to study the interactions of various factors. However, these factors are so numerous that a choice must be made. Given the present concern and knowledge, it is proposed to start a first set of experiments to study the interactions between ionizing radiations and electrophilic compounds, substances that have been shown, or are suspected, to be carcinogenetic.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. R. Devoret
Laboratoire d'Enzymologie
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Contract no.: BIO-E-426-81-F

General subject of the contract:

Genetic effects induced by radiation and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

Description of research work:

The heterozygosity for deleterious recessive mutations in human population may involve hundreds of genes and the somatic genetic risk of ionizing radiations may be principally due to the expression of these pre-existing mutations through aberrant mitotic segregations such as non-disjunctions and chromosomal breaks and rearrangements. Hereditary genetic load is caused principally by new mutations. Therefore this common programme proposes studies of both classes of these radiation-induced phenomena and in particular their prevention. (See also U.L.B. D.P. Radman contract n°BIO-E-420-B, C.N.R.S., Dr. Sarasin, contract n° BIO-E-427-81-F; C.N.R., Dr. Falaschi, contract n° BIO-E-428-81-I). When the genetic material of the cells of the body (somatic cells) or of the cells of reproductive organs (germ cells) is exposed to radiations or radiomimetic or carcinogenic chemicals, the following sequence of events ensues : after the lesions are produced, a signal is expressed ; this triggers various processes of repair and recombination (e.g. chromosomal rearrangements) involving diverse forms of DNA synthesis that will eventually restore a functional DNA structure with the original or modified nucleotide sequence. In bacteria the mutagenic effect of radiations and of chemical carcinogens is dependent upon the inducible error-prone DNA repair system. A similar pathway operates in mammalian cells and it may also involve an inducible system that generates chromosomal aberrations. All inducible phenomena are in principle preventable. The resistance to radiation displayed by a bacterial cell such as E. coli depends on the interplay of a score of enzymes governed by as many genes. Note that the recA protein plays a key role in the cellular resistance to radiations. The recA protein not only controls directly most repair processes (among which inducible error-prone repair : SOS repair) but it is also essential to basic cellular functions such as DNA replication, recombination, cell division and the induction of dormant viruses. The recA protein may protect DNA by binding to it and permit other enzymes to repair the damaged DNA. The elucidation of the functions of the recA protein in E. coli can provide a model for the understanding of basic questions concerning the radiation biology of mammalian cells. The cellular level of the recA protein, normally relatively low, is dramatically increased after DNA damage by radiations and radiomimetic chemicals. This important fact raises many questions, one of which being : what is the SOS signal ? The questions to be resolved are

the following:
1. DNA damage determines the amplification of the recA protein. To determine the respective parameters of this amplification it will be studied whether a) the amplification of the protein is in direct proportion to the DNA lesions; b) the inactivation of the lexA protein which normally represses the synthesis of the recA protein is in itself sufficient to bring about the amplification of the recA protein ; c) the role of the recA protein in the control of its own amplification.
2. DNA damage leads to the activation of the recA protein. Not only does the recA protein protect damaged DNA by binding to it but it seems to have a proteolytic action. This action might be necessary to remove from the DNA proteins that normally bind to DNA such as various repressors which may hinder repair and recombination enzymes to act on DNA. It appears that the recA protein is required for the proteolytic cleavage of repressors such as the lexA protein or the λ repressor. To determine the parameters which control activation of the recA protein we will study whether a) the recA gene codes for a proteolytic site in the protein ; b) a specific DNA lesion activates the recA protein ; c) a specific gene product is required for the activation of the recA protein to act as a protease.
3. The crucial importance of DNA damage at the origin of replication of the chromosome. It has been recently discovered that an adequate piece of DNA carrying the origin of replication when introduced in a cell leads to cleavage of a viral repressor. It is extremely important to investigate the mechanism whereby a) a specific site or a gene product located on or produced near the origin of replication leads to the activation of recA. In other words, how is the SOS signal produced ? b) the repressor of the recA protein is inactivated under these conditions similarly to a viral repressor; c) the activation and the amplification of the recA protein are necessary for SOS repair.
4. The role of gene products other than the recA protein in inducible error-prone repair. It has to be known : a) the functions of the gene uvm that controls SOS repair in connection with the recA protein. b) the functions of the infA gene in DNA replication, cell division and the proteolytic activity displayed by the recA protein. The methods used will combine the genetic techniques of molecular cloning of genes with biochemical techniques, namely titrations of the recA and repressors protein by immunoprecipitation and electrophoresis. Experiments involving radiation - and chemically - damaged DNA vectors carrying specific origins of replication will be performed. The various recA mutants isolated during the course of the contract will be used.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
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Contract no.: BIO-E-398-81-F

General subject of the contract:
Radiation induced and transmissible chromosome aberrations : Comparative sensitivity of mammals, risk assessment in man and interference of genetically radiation - sensitive constitutions.

Description of research work:

The research programme comprises three projects :
1. determination of the extent to which the results obtained experimentally with animals can be extrapolated to human pathology.
The karyotypes of over 100 species of mammal were compared with that of man. This analysis will be extended. The chromosomal evolution already established shows that specificity exists in the types of chromosomal changes accumulated. This suggests that chromosomal mutagenesis can be qualitatively, and not only quantitatively, different from one group to another. A cell bank of more than 70 species has been set up. This will serve as a source from which cells of different species, considered to be characteristic, can be drawn for irradiation. This irradiation should indicate whether or not the same types of modification are induced with the same frequencies from one species to another, which appears to be a necessary preliminary for any valid extrapolation. Moreover, if a specific mutagenesis is discovered, fundamental conclusions could certainly be drawn on that basis with regard to the mechanism of chromosomal rearrangements. This project, the first part of which is already much advanced as regards the primates, will be extended to other mammals. The part concerning the irradiation has barely begun. It will thus necessitate performing hundreds of chromosome analyses with good banding.
2. attempt to determine the actual risk to progeny of radiation-induced aberrations.
The likelihood of an abnormal infant being born after irradiation as the result of chromosomal aberration can be calculated on the basis of the following three factors : 1. survival of cells carrying a rearrangement. 2. risk of inadequate segregation of the rearranged chromosomes. 3. survival of any infants carrying an imbalanced chromosomal rearrangement.
Many of these factors can be assessed by means of a qualitative study of the radiation-induced rearrangements and a comparison with those that occur in human pathology.
Item number one will be studied on the basis of lymphocyte irradiation and of the analysis of various types of modification and their continuance through a number of cell generations. Item number two will be dealt with through the analysis of the segregation of chromosome rearrangements in human pathology. Item number three, on which work is about to be completed, will be supplemented by a study of the

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rearrangements observed in accidentally irradiated subjects.
3. attempt to identify subjects of high chromosomal sensitivity in the population.
Recent studies have demonstrated the existence of specific rearrangements in ataxia telangiectasia. The same rearrangements seem to exist in the parents, supposed to be radiation sensitive and heterozygotic for the abnormal gene.
The improvement of a direct and specific cytogenetic test could result from a thorough research, which will be extended to several families with affected children and to control samples.
In addition, various factors likeley to increase the frequency of specific anomalies or to make them appear will be tested.
Lastly, other diseases where a defect in DNA repair may occur will be investigated.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. U. H. Ehling
Institut für Genetik
GSF
Ingolstädter Landstrasse 1
D-8042 Neuherberg

Contract no.: BIO-E-395-81-D

General subject of the contract:

Radiation-induced gene mutation in mice.

Description of research work:

Attempts to detect radiation-induced genetic damage in man have, so far, proved inconclusive. Consequently, quantitative estimates of genetic hazards are still based on results from other organisms, primarily the mouse. Even though the point estimates of genetic effects in man have shown no significant increase over controls, the upper confidence limits of these provide at least some basis for comparison with the mouse results. In the experimental work on the mouse, emphasis has been placed on the effects of basic physical and biological factors that affect mutagenesis. These include the effects of radiation dose, dose rate, dose fractionation, radiation quality, age at exposure, and the interval between irradiation and conception. The differences in response of the two sexes, of the different germ-cell stages, and of various gene loci have also been investigated, along with dependent variation in the relative frequencies of viable mutations, lethals, small deficiencies, and major chromosomal aberrations. These important results for the estimation of the genetic risk were obtained with the specific locus method in which recessive visible mutations are studied. Regardless of how many markers can be practically combined in a specific locus test stock, the number of loci sampled is still only a very small fraction of the total genome. Results are therefore not as suitable for estimating total genome mutation rates as they are for comparing mutation rates obtained under different circumstances of exposure. Because the markers are chosen for ease of detection and for other practical considerations, they do not constitute a representative sample with regard to effects of mutations on the well-being of the population. It is the aim of the proposed programme to develop methods for the detection of mutations in mice which are directly comparable with hereditary diseases in man. This laboratory has been interested in the development of tests which screen for dominant mutations in mice. Such a system is advantageous because it does not require specific genotypes of the test animals and such a test system is directly analogous to induced deleterious mutations which are expressed immediately in human populations.
The spontaneous and radiation-induced cataracts can be easily characterized. The penetrance and the expressivity of the mutations will be determined and the fertility of the mutants carefully analysed. Until now it is not known if the genetic background of

mammals can have a pronounced effect on the radiation-induced mutation frequency. The cataract system provides an easy method to investigate the relationship between the radiation-induced mutation rate and the genetic background in mammals. The aim of the proposed programme for the investigation of radiation-induced dominant cataracts in mice is :

1) The determination of a representative mutation rate for radiation-induced genetic damage in mice.
2) The investigation of the influence of the genetic background on the radiation-induced mutation frequency in mice.
3) The quantification of the induced genetic damage in the first generation.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Finseninstitutet
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Contract no.: BIO-E-455-81-DK

General subject of the contract:

DNA repair capacity in patients with multiple malignant tumours.

Description of research work:

A theory for carcinogenesis states that injured cells which are able to repair a sufficient amount of DNA damage to survive and multiply but which still have erroneously repaired defects may be the origin of malignant tumours. This implies that DNA repair defects could increase the risk for cancer in a given individual as evident from a number of inherited diseases with repair defects all characterized by very marked symptoms in homozygous individuals. The significance of the heterozygote state is however unknown and so far only few attempts have been made to detect such populations. It has been shown that lymphocytes from patients with actinic keratosis have a decrease in DNA repair activity measured as unscheduled H^3-thymidine incorporation. A preliminary study in this laboratory has furthermore shown that the spread of unscheduled DNA repair synthesis is remarkably larger in lymphocytes from patients with multiple skin cancer than in a control material both studied after UVR irradiation. Ionizing radiation has so far not been studied. On the basis of these observations attempts will be made to study series of cases with multiple malignant tumours for the DNA repair capacity in relation to a number of damaging agents using both the gross determination of DNA repair activity and a number of more precise estimations of the single enzymatic processes involved in the phenomenon. The following populations will be analysed together with other comparable populations :

1. Multiple skin tumours where a large group of patients is being followed at the Finsen Institute.
2. Patients treated for cervix cancer some 10 to 15 years ago who were included in the huge study of leucemia induction after this treatment. The goal is to study the occurrence of secondary cancers. A large group of patients from Denmark was introduced into the study and can be reevaluated if alive.
3. Any other group of persons irradiated with ionizing radiation with or without chemotherapy where second cancers are reasonably common.
4. As a negative group, for evaluation of the occurrence of DNA repair effectiveness, diabetic patients where malignant tumours according to all acceptable publications should be low.

The group is furthermore attractive in so far as a large series where HLA tissue typing has been performed is under constant control in

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Copenhagen at the Niels Steensen Hospital and available for study. The types of radiations studied will be U.V., X rays or ionizing radiations of other energies. Cultures of lymphocytes will, in all cases, be used as experimental material but attempts will also be made, in smaller series, to correlate lumphocyte results to results obtained with other cells, for instance fibroblasts, from the same individual.

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Head(s) of research teams(s):

Contract no.: BIO-E-428-81-I

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General subject of the contract:

Genetic effects induced by radiation and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

Description of research work:

The heterozygosity for deleterious recessive mutations in human population may involve hundreds of genes and the somatic genetic risk of ionizing radiations may be principally due to the expression of these pre-existing mutations through aberrant mitotic segregations such as non-disjunctions and chromosomal breaks and rearrangements. Hereditary genetic load is caused principally by new mutations. Therefore this common programme proposes studies of both classes of these radiation-induced phenomena and in particular their prevention. (See also C.N.R.S., Dr. Devoret, contract n° BIO-E-426-81-F, C.N.R.S., Dr. Sarasin, contract n° BIO-E-427-81-F; U.L.B., Dr. Radman, contract n° BIO-E-420-81-B). When the genetic material of the cells of the body (somatic cells) or of the cells of reproductive organs (germ cells) is exposed to radiations or radiomimetic or carcinogenic chemicals, the following sequence of events ensues : after the lesions are produced, a signal is expressed ; this triggers various processes of repair and recombination (e.g. chromosomal rearrangements) involving diverse forms of DNA synthesis that will eventually restore a functional DNA structure with the original or modified nucleotide sequence. In bacteria the mutagenic effect of radiations and of chemical carcinogens is depending upon the inducible error-prone DNA repair system. A similar pathway operates in mammalian cells and it may also involve an inducible system that generates chromosomal aberrations. All inducible phenomena are in principle preventable.

1) Enzymes of DNA metabolism. The characterization of the DNA dependent ATPases and DNA binding proteins of animal cells will be continued. Attempts at obtaining mutant cell lines resistant to Aphidicolin, an inhibitor of DNA polymerase alpha, will be pursued. The production of monoclonal antibodies against terminal deoxynucleotidyl transferase will be attempted.

2) Subcellular DNA repair systems. The possibility that permeabilized cells, isolated nuclei and isolated chromosomes may provide assays of DNA repair synthesis will be investigated, with the aim of fractionating the soluble proteins in this process.

3) Study of subjects at high risk towards ionizing radiation. The detection of appreciable levels of terminal deoxynucleotidyl transferase in normal persons will be investigated as a possible indication of pre-leukemic state and hence high risk towards radioactive environment.

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4) Study of the inhibitors of DNA replication and repair in human cells. The mechanism of action of Aphidicolin, its properties and possible applications will be investigated : mechanism of inhibition of human and viral DNA polymerases, effect on repair synthesis and possible use as an anticancer drug.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. A. Farulla
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Via Cuboni 10
I-00197 Roma

Contract no.: BIO-E-401-81-I

General subject of the contract:

The problem of individual radiosensitivity in radioprotection : the use of radiosensitivity-tests for the screening of heterozygotes for the "chromosomal instability" syndromes.

Description of research work:

The rate of individual genetically determined radiosensitivity represents a very important and in the same time an intriguing point in the radioprotection, which is based upon the assessment of the "standard risk". In fact, in the individuals showing an increased radiosensitivity, the margin of prevention settled for the workers taken on the whole, could become inadequate and the risk inacceptable in the individual case.

A few genetic diseases transmitted as autosomal recessive traits are characterized by "chromosomal instability" and increased susceptibility to develop some types of cancers. These disorders which include the Louis-Bar syndrome or ataxia-telangiectasia (AT); Fanconi's anemia (FA), the Bloom's syndrome (BS) and xeroderma pigmentosum (XP), show peculiar chromosome aberrations similar in many instances to those induced in normal subjects by ionizing radiation, chemicals and viruses. The relatively high frequency in the general population of the genes responsible for these diseases, and the demonstration of an increased susceptibility to mutagens and to develop cancer not only in the homozygotes, but also in the heterozygotes for these diseases stress the importance of the identification of the heterozygotes for such genes.

It could be estimated that carriers of AT could be at least between 1 in 150 and 1 in 100 and those for FA 1 in 300. Taking XP, BS, AT and FA together, 1 in 50 person at the very least may be a carrier of one of the genes in question. For the carrier states of AT and FA, increased risk of malignancy ranging from 2 to 12 times the standard risk was implied. Thus the identification in the general population of the individuals carrying one of these genes becomes a task of major interest both in the field of preventive medicine and in particular in the field of radioprotection.

In a previous study on a group of patients with the AT syndrome an increased rate of lymphocyte radiosensitivity in vitro and an increased frequency of spontaneous chromosome breaks in the obligate heterozygotes for the AT gene have been shown. The main aim of the research programme is to develop laboratory tests capable to identify the heterozygotes for AT and FA genes. The study will be performed on the relatives of patients affected by these diseases, which at present are under control. In particular the research will try to assess the

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are under control. In particular the research will try to assess the dose-effect curve in vitro for graduated ionizing radiation and to identify the radiation dose capable to distinguish the heterozygotes for the AT and FA genes and the controls. The availability of a reliable and reproducible laboratory test of this kind will be of practical usefulness in radioprotection.

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Head(s) of research teams(s):

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Contract no.: BIO-E-410-81-B

General subject of the contract:

Radiobiology of mitochondrial DNA.

Description of research work:

It is a necessary prerequisite for the understanding of radiation damages of mammalian mitochondrial DNA, to put a major effort in the study of the repair of radiation damage of mitochondrial DNA in yeasts. At the present state of knowledge, priority must be given to the full identification of the different repair pathways and of the number of steps which are included in each pathway. Two laboratories will collaborate in this research program. The "Laboratoire de Biologie de la Fondation Curie de l'Institut du Radium à Orsay (Dr. E. Moustacchi) which in 1970 has obtained the first yeast strains deficient in the repair of UV-induced lesion of mitochondrial DNA and the "Laboratoire d'Enzymologie de l'Université Catholique de Louvain à Louvain-la-Neuve (Dr. A. Goffeau) which in 1979 has obtained a first set of strains deficient in the repair of gamma-rays induced lesion of yeast mitochondrial DNA. In contrast with UV, ionizing radiations were found not to produce extensive degradation of the mitochondrial DNA measured by the production of the rho^- mutation in the wild type. However, it has been possible to isolate mutants which produce rho^- and thus extensive degradation of mitochondrial DNA after gamma-irradiation. This demonstrates that efficient mechanisms exist for repair of gamma-ray-damaged mitochondrial DNA and fully warrants the feasibility of the present research proposal which is to obtain and to characterize a saturating number of thermosensitive mutants affected in the repair of gamma-ray-damaged mitochondrial DNA. After thorough characterization of the genetic properties of a large number of mutants by standard techniques (inheritance and gene segregation, complementation, mutability, survival, respiratory activities) the mutants will be classified either in the repair pathways common to nuclear and mitochondrial DNA damages or in the pathways specific for the repair of mitochondrial DNA. It will also be determined whether pathways for repair of gamma-rays damages are totally distinct from those involved in the repair of UV damages. It will then be attempted to determine what are the major mechanisms acting in mitochondrial repair : excision repair, recombination or other error-prone or error-free repair mechanisms.

The search for mutants deficient in recombination between the mitochondrial markers would be of great interest for testing different model mechanisms for recombination and of the involvement of this

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process in repair. In a further step, the most representative mutants will be selected in order to characterize their enzyme deficiencies. It was decided to concentrate on mitochondrial deoxyribonucleases :

1. to determine how many different activities can be detected in yeast mitochondria;
2. to determine the role of the deoxyribonucleases in repair by using mutants deficient in the repair of mitochondrial DNA.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. Dr. I. Hansmann
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Contract no.: BIO-E-393-81-D

General subject of the contract:

Mechanisms of non-disjunction.

Description of research work:

Based on the results of studies already carried out in the former programme, the investigations are concerned mainly with the mechanisms of radiation-induced chromosomal non-disjunction in female meiosis. In addition, after standardization of the methods of in vitro fertilizing human sperm with mammal oocytes, it is intended to carry out a comparison of the radiation-induced genetic effects on male germinative cells and on somatic cells.

a. Mechanisms of radiation-induced non-disjunction

On the basis of two models derived from experiments with animals with a high spontaneous frequency of genome mutations in ovulated ova, the question of the dose effect on genome mutations in a hamster and a mouse sub-line is to be investigated . At various stages of the late follicle maturation phase, mice and hamsters will be irradiated once with various, mainly low, X-ray doses and the effects of these doses on the high spontaneous rate of chromosomal non-disjunction in oocytes after stimulated ovulation will be investigated. In addition, a study will be made of the way in which frequent irradiation of young and old females with low X-ray doses affects the genomal mutation rate (direct X-ray effect). Further cytogenetic investigations with ovulated ova are to be carried out for determining whether there is any indirect X-ray effect on the well-known phenomena of increasing non-disjunction in ageing ova. By irradiating very sensitive ovaries in pre-pubertal mouse females, it is possible to reduce the overall population of primary follicles through cell death. Individual groups of females irradiated in this way will then be investigated at various ages for the frequency of non-disjunction in spontaneously ovulated oocytes. Comparison with the results obtained with non-irradiated females should show whether the radiation-induced reduction of the follicle population during infancy should be expected to give rise, later in adulthood, to a much earlier risk in later life of non-disjunction in the ovulated oocytes.

b. Radiation-induced genetic effect on male germinative cells and human somatic cells.

The radiation-induced genetic effect on human spermatogenesis is to be investigated with the method of fertilizing human sperm and mammal oocytes in vitro. For this purpose, haploid genomes from the sperm of men who have been obliged to undergo radiation therapy for medical reasons are to be analysed cytogenetically. Special consideration

will be given in this connection to a possible variation in sensitivity to X-rays between premeiotic, meiotic and post-meiotic germinative cells. Through simultaneous chromosomal analysis of somatic cells, e.g., cultivated lymphocytes, a relationship can be established between the radiation-induced genetic effect on somatic cells and on human germinative cells.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. J.A. Houghton
Department of Microbiology
University College of Galway
IRL-Galway

Contract no.: BIO-E-415-81-EIR

General subject of the contract:

Radiation repair processes in cyanobacteria and effects of radiation on the chromosomes of human gametes.

Description of research work:

The early stages of the first project shall involve a detailed investigation of both dark repair and photoreactivation in Gloeocapsa alpicola. Photoreactivation has been found to be extremely efficient in this species and so these studies will include an examination of the biochemistry and kinetics of this repair process and an investigation of its genetic control and regulation. Dark repair processes utilizing a system similar to excision repair in E. coli has now been demonstrated and these studies will continue on the enzymology of this system to establish its mechanism and its role in the evolution of eukaryotic repair. In view of the importance of cyanobacteria in the evolution of higher organisms, this study should provide valuable information on the evolutionary development of radiation repair mechanisms. These studies will also include a detailed investigation of the radiation repair process first described in the cyanobacterium Anacystis nidulans. These studies have revealed that this system, which is more efficient following pre-incubation in the dark or under anaerobic conditions in the light, is also present in Gloeocapsa alpicola. It has been suggested that this system may be unique to the cyanobacteria and these studies will concentrate on establishing the biochemistry and enzymology of the pathway as well as assessing its contribution to radiation repair and to the development of radiation repair processes in other organisms.

In recent years there has been considerable interest in the study of extra-chromosomal plasmid DNA and the incorporation of chromosomal genes into these plasmids. Within the last year the presence of plasmids in cyanobacteria has been reported and chromosomal genes from E. coli have been incorporated into the plasmids which have then been mobilized. Work is already underway in this laboratory on the plasmid DNA of Gloeocapsa alpicola. The major incentive to this study has been the suggestion that many of the genes determining radiation repair processes may be carried by plasmid DNA. The study of the role of cyanobacterial plasmids in the control of radiation repair will thus be a major priority in this research project. The function of plasmid DNA in radiation repair will be studied and it is hoped to mobilize these genes using genetic engineering techniques. It is then undertaken to attempt the transfer of these genes into other species and into other microbial groups. This study will also be complemented by the use of genetic transformation procedures for transferring the

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genes for radiation repair to other groups. In the second project the effects of radiation on the induction of non-disjunction and other chromosomal aberrations in the gametes of man will be investigated directly. In the first year of the contract the techniques developed for the direct study of the chromosomes of human spermatazoa by fusion with zona pellucida free eggs from golden hamsters will be utilized and exploited. The correlations between a wide range of factors on the incidence of non-disjunction in male meiosis will be studied. The effects of radiation on the chromosomes of sperm will also be investigated. A major emphasis of the project will concern the study of the cytogenetics of sperm from males who have received irradiation for radiodiagnostic or radiotherapeutic reasons. Studies will also be carried out on the chromosomes of sperm from men who live in areas with different levels of background radiation. These studies will significantly contribute to our understanding of the role of radiation in the induction of aneuploidy and chromosomal aberration in man. Furthermore, direct irradiation experiments will be carried out using experimental animals. The effects of gonadal irradiation in both males and females may be determined using a modification of existing techniques. The effects of ovarian irradiation of hamsters on the incidence of aneuploidy in the egg chromosomes can be studied by fertilizing eggs from irradiated hamsters by sperm from normal human males or males from other species. Any abnormalities in the hamster egg chromosomes induced by the irradiation may then be recognized. Similarly, males from another species e.g. rats, mice, or other suitable animal may also be subjected to different doses of gonadal irradiation and the fertilization of normal hamster eggs by the sperm of these irradiated males will permit a direct assessment of the role of irradiation on the induction of chromosome aberration. This project will enable the direct study of the contribution of radiation towards the incidence of non-disjunction and chromosomes rearrangement in man and, as such, will be a very useful component of the Radiation Protection Programme.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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und Strahlenbiologie
Martinistrasse 52
D-2000 Hamburg 20

Contract no.: BIO-E-391-81-D

General subject of the contract:

Study of the relationship between DNA-strand breaks, chromosome aberrations and loss of the proliferation capacity of mammalian cells.

Description of research work:

The objective of the research project is to investigate the relationship between DNA-strand breaks and chromosome aberrations in mammalian cells exposed to ionizing radiation and their importance for the loss of proliferation capacity. The research is to be carried out on synchronized cells, on cells that continue to proliferate and on cells that cease to proliferate after irradiation. The investigations are to be conducted with Chinese hamster ovary cells (CHO), the DNA-strand breaks being measured by means of hydroxyl-apatite chromatography, while the chromosomal aberrations will be determined with light and fluorescent microscopy and the survival rate by means of the colony test.

The number of strand breaks occurring after irradiation, their repair kinetics and the extent of repair are to be determined as a function of different phases of the cell cycle. The cells will be treated with X-rays and 14-MeV neutrons as well as with internal beta-radiation (^{3}H-thymidine incorporated in DNA). After identical treatment, the survival rate and the number of chromosome aberrations will be determined.

Following irradiation, the cells can be classified in two populations characterized by different proliferation capacities. After a certain delay, one portion of cells proliferate at the same rate as before whereas the growth rate of the remaining portion decreases exponentially with time. In this context, the extent to which cells that proliferate and those that no longer proliferate after irradiation differ in the amount of DNA-strand breaks repair and the number of chromosome aberrations will be investigated.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. J. Kiefer
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Univ. Giessen
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D-6300 Giessen

Contract no.: BIO-E-392-81-D

General subject of the contract:

Mutation induction and biochemical damages by heavy-ion and low dose-rate gamma-irradiation.

Description of research work:

It is the aim of the research programme to contribute to the understanding of mutation induction at the cellular and the molecular level. The test object is the yeast Saccharomyces cerevisiae which offers unique possibilities. It can be studied in different states of ploidy, many repair deficient mutants are available as well as cell cycle mutants, and radiation induced alterations can be studied biochemically within the same dose range as survival. It can also be grown for long periods in continuous cultures which makes it particularly suitable for studies with low dose rate irradiations.
The dependence of radiation induced mutation on dose rate is also of considerable importance for radiation protection. Most of the experiments reported so far demonstrate a reduced yield with decreasing dose-rate but involved only comparatively short exposure times. In the study suggested continuous cultures shall be employed subjected to long term low dose-rate gamma-irradiation (several days) provided by Co-60 gamma-source. It was previously shown that this experimental system attains a stationary state under irradiation representing essentially a long term exponential culture. Thus it is possible to investigate mutation induction by low dose-rate exposure under stable experimental conditions. Such studies will be carried out within the frame of the present contract.

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Head(s) of research teams(s):

Dr. H.P. Leenhouts
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Contract no.: BIO-E-409-81-NL

General subject of the contract:

An investigation to quantify the effect of synergism between radiation and other mutagenic agents at low radiation doses in eukaryotic cells.

Description of research work:

The investigations proposed in this project are concerned with determining how a radiation dose relationship is altered in the presence of different mutagenic agents and emphasis will be placed on the changes occurring at low doses. As the programme progresses attempts will be made to associate molecular processes with the alteration in the dose relationship. The induction of somatic mutations in the stamen hairs of Tradescantia has been chosen as the biological end-point for experimentation.
The radiation dose relationships for mutation induction will be determined in the presence of a different mutagenic agent.
Parameters which will be considered are concentration or "dose" of the mutagen, dose rate and quality of the radiation, time between the treatments, and order of the treatments.

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Head(s) of research teams(s):

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Contract no.: BIO-E-451-81-B

General subject of the contract:

Experimental studies on the teratogenic effects of small doses of radiation delivered during the preimplantation period.

Description of research work:

It is proposed to study in detail and by multidisciplinary approach the effects of low doses of X-irradiation on the embryo during preimplantation. The in vitro techniques together with cytogenetic and ultrastructural observation methods will be used to determine the causes of embryonic mortality, which is the most common consequence of an exposition to X-irradiation at this time. The claim of certain classical but already old investigations that doses of only a few rads provoke serious lesions in the embryo can also be substantiated in this way. Female mice will be irradiated at different stages of pregnancy. Some will be sacrificed one day before delivery, and their uterus and ovaries (corpora lutca) will be examined to determine the preimplantation mortality resulting from irradiation. The other females will be sacrificed directly after irradiation, and their embryos will be flushed out from the oviducts and cultured in Brimster's medium. At the end of this first culture, embryos having reached the blastocyst stage will be transferred into modified Eagle medium for a period of 4 days, to examine their ability to implant and differentiate their inner cell mass into ectoderm and endoderm. Comparison with observations on unirradiated embryos will render possible to determine if the preimplantation mortality induced by irradiation is due to a perturbation of the first embryonic divisions following irradiation or to an impairment of implantation into the uterus. In the later case, a comparison of the results of the in vitro and in vivo experiments will allow to distinguish between direct effects on the embryo and indirect effects via the mother (hormonal perturbation).
Cytogenetic abnormalities will be followed at different stages of development of normal and irradiated embryos, with the technics elaborated by Tarkowski, in order to precise the role of chromosomal anomalies in radiation induced perturbations of the embryonic development.
The ultrastructure of the embryos will be studied to detect any structural abnormality caused by radiation.

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Head(s) of research teams(s):

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Contract no.: BIO-E-403-81-NL

General subject of the contract:

The genetic and biochemical basis of radiation sensitivity in human and other cells in culture.

Description of research work:

The purpose of the project on the genetic analysis of DNA repair is the identification of genes involved in the repair of UV dmage in eukaryotic cells, in particular those of human origin. To this end recombinant DNA techniques will be used, as well as other methods by which genetic material can be transferred from one species to the other. The ultimate aim is the cloning of mammalian - if possible human - genes involved in the repair of DNA damage, in a suitable micro-organism (preferably E. coli) and the characterization of their gene-products. Before experiments of this nature can be attempted with a reasonable chance of succeeding, several barriers should be overcome, which necessitates a number of introductory studies.

The project will involve :

a. The transfer of repair genes of micro-organisms to (repair deficient) eukaryotic cells and study of their expression and the effect on the repair characteristics of the host.

b. The development of general techniques for cloning eukaryotic genes in E. coli and, possibly, in yeast, including the development of hybrid cloning vectors.

c. The development of vectors suitable for cloning in mammalian (human) cells.

d. The application of the techniques sub b and c for the cloning and - if possible - expression of (human) repair genes in micro-organisms.

(In this project a close cooperation is foreseen with the State University of Leiden, Prof. Dr. A. Rörsch, contract n° BIO-E-407-81-NL and with Prof. Dr. D. Bootsma, Erasmus University, Rotterdam, contract n° BIO-E-404-81-NL.

The project on the biochemical analysis of DNA repair aims at the identification of various DNA lesions in mammalian cells and at the elucidation of their repair. In the previous contract repair pathways in mammalian cells were studied extensively, with special attention for the defective pathways in mutant cells of human origin. It could be demonstrated that the understanding of error prone/error free pathways will be greatly furthered when specific pathways associated with different (and characterized) lesions could be identified. In this project a number of methods will be used to study the pathways that are followed in mammalian cells for the repair of identified lesions. The methods include :

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a. Measurement of repair replication by density gradient centrifugation;
b. Measurement of unscheduled DNA synthesis by autoradiography;
c. Measurement of repair DNA synthesis by the BUdR-313nm light method;
d. Measurement of the presence (and disappearance) of certain lesions with the use of enzymes (specific endonucleases);
e. Idem, with the use of labeling techniques (lesion induced by radioactively marked chemicals);
f. Idem, with the use of methods detecting DNA/DNA or DNA protein crosslinks;
g. Idem, with the use of specific immunological detection methods (in this approach the application of "hybridoma's" will be attempted in collaboration with the Erasmus University, Rotterdam, Prof. D. Bootsma, contract n° BIO-E-404-81-NL.

If during the period of the contract one or more mammalian repair genes can be cloned in a micro-organism and can be brought to expression (see first project), it will be attempted to isolate and characterize the gene product(s), in particular with regard to the function in repair pathways.

In the project on the consequences of DNA damage and repair the attention is focused on the individual sensitivity of human cells towards ionizing radiation. As is stated in the preamble, there is a growing concern about the variability in the repair capacity of the apparently "normal" human population. Sofar the main approach to investigate this problem has been a study of the survival characteristics of cultured primary human cells. However, for an extensive screening of large groups of the human population this method is too laborious. It is our aim to adapt more simple, fast and sensitive biochemical techniques to obtain data that are relevant for the radiosensitivity of cells. This will involve the development of techniques by which various types of radiation damage in the DNA of mammalian cells can be reliably detected.

h. Measurement of the presence (and disappearance) of single-strand breaks and double-strand breaks with the very sensitive techniques of alkaline and neutral elution, enables the detection of breaks after irradiation at biologically relevant doses.

The alkaline elution technique will also make it possible to follow the appearance and disappearance of incision breaks during repair of some radiation induced lesions. This will be very helpful in elucidating the mechanisms of repair, in particular when the effects of specific repair inhibitors are also studied.

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Head(s) of research teams(s):

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Section Biologie
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Centre Univ. - Bât. 110
F-91405 Orsay

Contract no.: BIO-E-397-81-F

General subject of the contract:

Nuclear and mitochondrial genetic lesions induced by radiations in a lower eukaryote : mutagenic and recombinogenic processes in relation to replication and repair.

Description of research work:

In this research programme the yeast Saccharomyces cerevisiae will be used as a model eukaryotic system. It will be taken advantage of three main features of this organism : a) this yeast is not a strict aerobe and the mitochondrion can be dispensed with; cells with limited or mitochondrial DNA alterations can still survive; b) a number of well defined repair-deficient mutants (rad) are available and the fusion between such yeast cells and human fibroblasts can be performed ; c) mitotic and meiotic intra and intergenic recombination is induced by radiations in diploid cells in defined stages of DNA replication and the organism is amenable to biochemical analysis of recombinational steps.

I. Repair and mutagenesis of the mitochondrial genome. Mitochondrial mutations are of the deletion type followed by repetition of retained DNA sequences ("petites") or point mutations (ANT^R or mit^-). The first type of mutations are likely to be lethal in strict aerobes. The second class has been identified in mammalian cells in culture and the possible interference of such mutations with the functioning of repair systems is not excluded. Nuclear and mitochondrial mutations which favor the spontaneous and UV or X-rays induced mutations of both types have been identified. On the other hand, it appears that the sensitivity of the mitochondrial genome differs according to the DNA sequence and/or function. Making use of the DNA restriction enzymes techniques applied to pulse labelled mitochondrial DNA it is planned to determine the replication and recombinational functions in relation to rescue of mitochondrial markers in cells treated by radiations. The process by which repetitive sequences can be generated will be also examined, the yeast mitochondrial genome being particularly favourable to analyse this important question. In order to define the interactions of the mitochondrial genome alterations on the cellular response to radiations nuclear genetic end-points will have to be examined in parallel. On a long range, the cloning of yeast genes involved in repair is envisaged.

II. Human cell - Yeast heterokaryons. Fusion between human cells and yeast protoplasts occurs in the presence of polyethylene glycol. The conditions for obtaining an optimal frequency of hybrids have been established. It is planned to examine by fusion between Xeroderma pigmentosum and wild type repair-competent yeast if complementation for repair functions can take place. If this is the case the collection

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of well defined repair mutants in yeast can be further used to classify the defects in human cells.
III. Analysis of the mechanism(s) leading to recombinogenic events. Genetic recombination occurs spontaneously in vegetative eukaryotic cells, and is efficiently induced by ionizing radiations. Because of the existence in different microorganisms of radiation sensitive mutants that are depressed for induced recombination, it is thought that the recombinational event shares common steps with a repair process. Therefore mitotic recombination is important not only for its genetic consequences but also because it leads to the repair of potentially lethal lesions. A genetic system was set up, using conditional cell cycle mutants (cdc), in which the recombinational events are forced to occur at specific mitotic stages. This system also offers the possibility to allow one round of DNA replication after irradiation and therefore to investigate the role of DNA replication in the recombinational process. The mechanism of induced recombination will be analysed making use of the cdc mutations coupled with rad mutations. The following questions will be examined : Does the repair and/or the recombination occur before or after DNA replication ? To what extent is DNA replication involved in the formation of recombinants ? Do sister-chromatid exchanges occur in yeast and how are they related to repair ? Since sister chromatid exchanges are genetically silent, they will be analysed biochemically using the heavy-light DNA techniques. Since previous results have shown that the recombinational process is induced by the presence of DNA lesions the kinetics of induction and of the disappearance of the recombinational competent state remains to be determined

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Head(s) of research teams(s):

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Contract no.: BIO-E-421-81-NL

General subject of the contract:

Development of in vivo mouse models for human X-ray sensitive diseases.

Description of research work:

It will be attempted to develop an in vivo mouse model for X-ray sensitive human diseases such as Ataxia telangiectasia, Fanconi's anaemia, Huntington chorea and some retinoblastomas. Most of these diseases are characterized not only by an enhanced sensitivity to the induction of chromosomal aberrations by X-rays, but also by many congenital neuroanatomical and/or physiological abnormalities. Among the hundreds of mouse mutants generated during the last decades there are several with congenital abnormalities similar to the ones observed in human radiosensitive patients and these mouse mutants will be selected for initial screening for radiosensitivity. The screening of the selected mutants will be performed with the bone marrow micronucleus test using exposures of 50 and 100 rad X-rays and sampling times of 18 and 27 hours. For each dose and sampling time it is planned to use two males and two females. This procedure will generate quick and reliable information about the chromosomal radiosensitivity of mutant versus normal laboratory mice. After initial screening and identification of some interesting mutants more detailed genetic and biochemical analyses will follow. With respect to genetic tests, chromosome breakage by metaphase analysis and study of sister chromatid exchanges in (a) bone marrow cells, (b) peripheral blood lymphocytes, and (c) spermatogonial cells, is planned, together with the study of sister chromatid exchanges, chromosome breakage and point mutations in cell cultures derived from the mutant mice and normal laboratory mice. Other tests that can be of interest are dominant lethals and translocation induction in stemcell spermatogonia. With biochemical techniques such as neutral and alkaline sucrose gradients and neutral and alkaline elution DNA repair processes of radiation induced single and double strand breaks can be studied. Both sparsely and densely ionizing radiations will be employed. The study of heterozygotes, in case of autosomal linked mutants, will also be considered. The choice of mutants at first instance will be brindled (Mo^{vbr}), fidget (fi), gyro (Gy) jimpy (jp), ochre (Och), sprawling (Swl), varitint-waddler (Va) and viable-brindled (Mo^{vbr}). Other possible candidates are ataxia (ax), nervous (nr), staggerer (sg) and weaver (wv) which can be studied at later stages. Of this collaborative research effort the MRC Radiobiological Unit at Harwell (see contract

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n° BIO-E-429-81-UK) will do the breeding of the mutants and part of the genetic test whereas the screening, the biochemical analysis and part of the genetic tests will be performed in the Department of Radiation Genetics and Chemical Mutagenesis in Leiden.

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Head(s) of research teams(s):

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Contract no.: BIO-E-475-81-I

General subject of the contract:

Studies of the molecular basis of post-replication repair.

Description of research work:

The field of DNA repair has recently received a surge of interest with the evidence that most types of repairable damage in DNA are both mutagenic and carcinogenic. This correspondence has been supported by the discovery that a deficiency in the repair of DNA photoproducts is correlated with the high incidence of sunlight-induced cancer in the human hereditary disease known as Xeroderma Pigmentosum (XP). Different types of repair are known. One of these is the post-replication repair that is present in bacteria as well as in mammalian cells. The molecular basis of this mechanism is not yet clear and new approaches are certainly required for a better understanding of this process.

It is possible to study the molecular basis of UV repair by the use of plasmids which are easy to analyze and to handle. The plasmid may be transferred into a genetically well defined host by conjugation and transformation. Application of gene cloning and DNA sequencing techniques allows the analysis of the gene structure and the study of its function. Moreover it is possible to transfer plasmid DNA in eukaryotic cells in order to observe the expression and function of plasmid genes. In the literature several plasmids have been described that are capable of affecting repair functions and mutagenesis. The aim of this programme is to study, using techniques of genetic engineering, the mechanism of action and regulation of post-replication repair. To this end it is proposed to characterize the gene of pR plasmid involved in error prone repair in E. coli.

The TP120 plasmid is known to determine enhanced UV survival in E. coli wild type and uvrB and polA mutants but not in recA mutants. The dependence of TP120 and RecA function suggest that this plasmid is exerting its effect through an interaction with the RecA dependent process. The study of the interaction of this plasmid with UV survival may clarify the molecular mechanism of the RecA dependent repair process. In this laboratory a smaller viable plasmid, named pR, has been obtained by restriction analysis of TP120. This plasmid contains the origin of replication as well as the genes responsible for ampicillin and UV resistance. The insertion of the Tn5 transposon in the pR plasmid allowed to select several pR::Tn5 plasmids in which UV resistance was inactivated by the transposition event. Comparison of protein synthesis in minicells containing the pR or pR::Tn5 plasmid showed that pR codes for a 22.000 m.w. protein, absent in the protein pattern of pR::Tn5. This protein may be the one involved in "error

prone repair" process in E. coli.
The work to be continued by this group will involve the following research lines:
a) analysis of the size of the gene that codes for the 22.000 m.w. protein by physical mapping with the Tn5 transposon. The size of the gene will be determined by the analysis of selected pR plasmids in which the insertion of Tn5(Km) transposon inactivates the UV resistance gene. In fact it has been shown that transposon Tn5, once inserted into a gene eliminates its functions thus inducing mutation by insertion. In this laboratory several colonies have been selected whose UV resistance was inactivated by transposition. In these colonies Tn5 is inserted in different sites of the plasmid gene that determine the UV resistance; by restriction analysis with appropriate endonucleases it will be possible to localize the transposon insertion sites in these mutants and consequently determine the size of the gene.
b) DNA sequence analysis of the gene involved in UV resistance. The analysis of the nucleotides sequence of this gene allows to determine the aminoacidic sequence of this gene and physico-chemical properties of the 22.000 m.w. protein previously identified. Moreover by analysis of the nucleotides sequence it is possible to obtain information about regulative signals (promoter and terminator) of transcription of UV repair gene.

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Head(s) of research teams(s):

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Contract no.: BIO-E-400-81-I

General subject of the contract:

Studies on induced chromosome aberrations for a better evaluation of genetic risk to man.

Description of research work:

The work will be focused on the mechanisms of formation of chromosome aberrations and their relations to those involved in meiotic recombination and in the production of sister chromatid exchanges (SCE) and on the relationships between gene mutation, chromosome aberrations and sister chromatid exchanges. One approach to gaining insight into the mechanisms of chromosome breakage and rejoining is genetic dissection by mutants affecting different aspects of DNA metabolism. In Drosophila melanogaster the past several years have witnessed a veritable explosion in the discovery of mutations that affect the metabolism of chromosomal DNA. Two classes of mutations, meiotic mutants and mutagen sensitive mutants have been shown to be rich sources of lesions in processes that are necessary for maintaining the integrity of mitotic chromosomes. Using these mutants a systematic dissection of the mechanism of chromosome breakage and rejoining in Drosophila has been undertaken. This project is aimed at the continuation and extension of these studies in the following directions :

a) characterization of the extant meiotic and mutagen sensitive mutants for their sensitivity to the induction of chromosome aberrations by ionizing radiations, methyl-methanesulfonate (MMS) and mitomycin-C (MCC);

b) study of the frequency of spontaneous and induced SCEs in the extant meiotic and mutagen sensitive mutants;

c) study of the frequency of spontaneous and induced chromosome aberrations and SCEs in opportune double mutants;

d) selection of additional mutants affecting somatic chromosome stability by screening for the presence of spontaneous chromosome aberrations : 1) X-linked temperature sensitive lethals; 2) autosomal meiotic mutants; 3) autosomal female sterile mutants: 4) autosomal temperature sensitive lethals. A substantial part of these mutants will be isolated in our laboratory after treatment with ethyl-methane sulfonate (EMS);

e) characterization of the newly isolated mutants for their effects on : 1) the frequency of spontaneous and induced chromosome aberrations and SCEs, 2) the frequency of spontaneous mutation; 3) meiotic and mitotic recombination and 4) mutagen sensitivity. An attempt will also be made to characterize some of the newly isolated mutants for their effects on DNA replication and on known DNA repair processes.

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Head(s) of research teams(s):

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Contract no.: BIO-E-450-81-I

General subject of the contract:

Relationships between chromosome structure and induced chromosome rearrangements.

Description of research work:

A series of observations have shown a non random distribution of chromosome aberrations and sister chromatid exchanges (SCE) along the chromosomes. For example, a higher incidence of chromosme aberrations in the heterochromatin has been observed in many organisms and heterochromatic-euchromatic junctions have been reported to be a preferential site for the occurrence of both chromosome aberrations and SCE's. The research aims at : a) a better definition of the sensitivity of different chromosomal regions to both chromosome aberrations and SCE's; b) an understanding of the molecular basis of the differential mutagen sensitivity of the various chromosome regions; c) obtaining some insight into the gentic control of the stability of different chromosome regions. These research lines are grouped into two projects :

1. Sensitivity to chromosome aberrations and SCE's of different heterochromatic regions and of euchromatic-heterochromatic junctions.

A series of recent chromosome banding techniques (Q-banding, Hoechst banding, N-banding, etc.) have shown that heterochromatic regions can be differentiated into blocks with different cytochemical properties reflecting different contents of AT-vs GC-rich DNA and a differential localization of chromosomal proteins. In order to investigate whether different heterochromatic regions (as defined by banding techniques) have different mutagen sensitivity, somatic cells of D.melanogaster, D.virilis and D.hydei will be treated with X-ray, UV-ray, methyl-methanesulfonate (MMS) and mytomicin-C (MMC) and scored for the regional distribution of induced chromosome aberrations. Somatic cells of the same Drosophila species will also be treated for two rounds of DNA replication with BUdR and scored for the localization of spontaneous and induced SCE's. In these studies it will be carefully investigated whether the regions at the interfacies of two different kinds of heterochromatin and the euchromatic-heterochromatic junctions behave as "hot spots" for both induced aberrations and SCE's. Since euchromatic-heterochromatic junctions of D.melanogaster exhibit a very high incidence of both aberrations and SCE's the relative sensitivity of eu-heterochromatic junctions of different chromosome arms will be studied as well as the sensitivity of artificially generated eu-heterochromatic junctions (the junctions generated in inversions and translocations having one heterochromatic breakpoint).

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2. Studies on the genetic control of the stability of different chromosome regions.

Several mutants of Drosophila melanogaster have been isolated which control the stability of specific chromosome regions. For example, mutations at the mus-105 locus produce aberrations preferentially localized in the euchromatin whereas the spontaneous aberrations scored in mus-109 are clustered in the heterochromatin. A similar situation exists in the human disease. Bloom syndrome where most breaks and exchanges are localized near the centromeric regions. Recently a mutant of D.melanogaster has been also isolated (cdm-302) that produces a high frequency of spontaneous breaks clustered in specific regions of the X and the Y chromosome. Taken together these observations clearly indicate that the stability of different chromosome regions is under specific genetic control. This project is aimed obtaining insight into the molecular basis of the regional control of chromosome stability. These ara two possible explanations for the clustering of chromosome aberrations in specific chromosome regions. Different chromosome regions may contain different spontaneous lesions which are repaired by the normal products of the loci identified by the mutations. Alternatively different chromosome regions may contain the same type of molecular lesions, which however, are repaired by different region-specific gene products : To discriminate between these two possibilities mutants at mus-105, mus-109 and cdm-302 loci will be treated with a variety of mutagens (X-ray, UV-ray, MMS, MMC, bleomycin, etc.) and the pattern of induced aberrations determined. If the first explanation were correct only a restricted class of related mutagens would be expected to produce a high incidence of aberrations with a specific regional distribution. However if the second explanation were correct a broad class of unrelated mutagens would be expected to produce aberrations clustered in the regions repaired by the normal alleles of these mutations.

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Head(s) of research teams(s):

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Contract no.: BIO-E-411-81-UK

General subject of the contract:

Radiation damage, repair and genetic change in eukaryotic cells.

Description of research work:

The Swansea group will undertake a series of 2 interrelated research projects upon radiation damage in yeast. The first project considers radiation damage and repair in yeast cultures undergoing mitotic and meiotic cell division. The radiobiology and yeast cultures can be readily studied during both mitotic and meiotic cell division events. Technical developments in the Swansea laboratory involving the use of both zonal and elution centrifuge rotors allow the isolation of large numbers of cells at specific stages of the cell cycle for radiation exposure and study. At the present time little information is available with regard to the activity of repair enzymes of yeast during meiotic cell division. Genetic studies in the Swansea laboratory have resulted in the construction of numerous diploid strains of yeast carrying defective alleles of the genes regulating DNA repair in mitotic cell division which may be used to study meiotic cell division. Studies will be made upon :

a) the relative sensitivity to ionizing radiation of repair proficient (RAD) and repair deficient (rad) yeast cultures during both mitotic and meiotic cell division.

b) the nature of DNA damage, both qualitative and quantitative during mitotic and meiotic cell division and the role of modifying treatments.

c) the biochemistry of DNA repair during mitotic and meiotic cell division and the characterization of the enzymes of DNA repair in yeast.

d) the relative contributions of constitutive and induced repair activity during cell division.

These studies are intended to provide detailed information on the nature of the DNA lesion, its repair and genetic control in meiosis.

The second project is a comparative study of genetic change in eukaryotic cells irradiated during mitotic and meiotic cell division.

A study will be made of a range of genetic endpoints induced by ionizing radiation in yeast cells undergoing cell division. Strains have been, or are in the process of construction, which allow the estimation of :

a) radiation induced forward and reverse mutations.

b) radiation induced inter- and intragenic recombination.

c) radiation induced chromosome aneuploidy.

Comparisons will be made of the relative frequencies of each genetic

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endpoint in cells irradiated in both mitotic and meiotic cell division in both repair-proficient and repair-deficient strains. Use will be made of both synchronous and asynchronous cell cultures. A detailed analysis will be made of the various components of repair in the induction of each of the separate genetic endpoints. In the case of study of mutation and recombination during mitotic cell division there will be close collaboration with Dr. Moustacchi's group in Paris who are using an essentially complementary procedure for the measurement of these parameters (contract BIO-E-397-81-F).
Particular emphasis will be placed upon the study of radiation induced chromosome aneuploidy and the development of a model system for the study of the detailed kinetics of changes in chromosome number after radiation exposure. The relationship between metabolism, repair activity, position in the cell cycle and the role of modifiers of induced aneuploidy will be determined.

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Head(s) of research teams(s):

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Contract no.: BIO-E-460-81-UK

General subject of the contract:

Studies in microdosimetry, cellular radiobiology and track structure.

Description of research work:

The basis of all radiological protection norms and risk estimates is a study of the biological effects of radiation. This also requires a knowledge of the basic physics of the interaction of radiation with matter. Starting with survival and mutation data from mammalian cells, microdosimetric models have been used to calculate for different radiations various parameters such as RBE, interaction diameters, etc. as a function of dose and dose rate. Fundamental to a full understanding of the biological effects is the study of the physical track structure of ionizing radiations. Thus the aim of this programme is to bring together the work on mutation and survival in mammalian cells and the study of track structure in a low pressure cloud chamber through the theoretical modelling using microdosimetric techniques. These techniques can then be used to calculate risk for various radiations and to establish RBE's and hence quality factors.

Energy deposition distributions will be calculated on both the micrometre and nanometre scales for gamma radiation, fast neutrons and tritium and other radionuclides such as I-125 and P-32 which may be taken into the body. The distributions will be determined by means of (1) a modification of the computer program of Edwards and Dennis, (2) the electron slowing down program of Holt, and (3) data obtained with a low pressure cloud chamber (more details are given in the technical description of contract BIO-A-306-81-UK). The distributions will be used to construct models for the response of each separate mechanism of radiation effect as found in the biological experiments, as a function of radiation dose, dose-rate and quality. The effects of repair processes will be incorporated in the models.

The energy deposition distributions will also be used to interpret published data on the radiation response of bacteria under various environmental conditions, since it is believed that success in interpreting these data will be a very useful guide in constructing convincing models for mammalian cells.

Co-operation will be established and maintained with similar programmes carried out by other laboratories in Europe.

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Head(s) of research teams(s):
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Contract no.: BIO-E-394-81-D

General subject of the contract:

Investigation of radiation induced damage to DNA in eukaryotic cell systems and its possible relationship to survival.

Description of research work:

The effectiveness of a radiation protection programme, taking into account the optimal uses of radiation in both medicine and industry, depends on a sound understanding of the radiobiology of eukaryotic cells. A full comprehension of the response of these cells to radiation depends in turn on a knowledge of molecular events, stemming from the interaction of radiation with living matter, which lead to death of injury resulting in disease. Probably the main but perhaps not the only target in the cell for critical radiation injury is the DNA. This is indicated by the occurrence of chromosome aberrations and supported by results showing an altered pattern of cell killing in cells treated with DNA binding or modifying drugs. It cannot however be ruled out that other subcellular structures play some secondary role in the response of cells to radiation. It is probable that most of the damage produced in the DNA by radiation is reparable but in spite of this a small fraction may remain unrepaired or irreparable. It is likely that this small unrepaired fraction of the damage is that which leads to chromosomal aberrations, cell death and disease of the irradiated organism. It is therefore important to investigate factors influencing the repair of critical lesions in the DNA and mechanisms leading to irreparable damage in the chromosomal material. The induction of chromosome aberrations by radiation indicates that DNA double-strand breaks as critical lesions, however base damage in DNA may also play some part in the cellular response. The repair and the residual number of DNA double-strand breaks in eukaryotic cells will be measured. Because of the vast size of the chromosomal DNA molecules in mammalian cells it is not possible, even using the most sensitive techniques, to measure less than about 100 double-strand breaks per cell. Thus if only one or a few remaining double-strand breaks are responsible for cell lethality, mammalian cells do not provide a suitable system in which to study a possible causal relationship. Yeast, on the other hand, provides an ideal system in which to investigate such a relationship. In this system, double-strand breaks as well as survival can be measured in the same dose range. It is possible to establish not only the total number of radiation induced double-strand breaks but also the number of these which are irreparable per cell. To investigate the possibility of a causal relationship between double-strand breaks and cell death, the repair of double-strand breaks and cellular repair will be investigated under

exactly the same conditions. From these data and on the assumption that one or a few double-strand breaks are lethal for a cell, hypothetical survival curves will be calculated and compared with experimentally established survival curves. To investigate the role of double-strand break repair in cellular repair substances will be used which are known to influence survival of irradiated cells. In addition to unrepaired double-strand breaks, misrepaired double-strand breaks (chromosome aberrations) may also be a lethal event for the cell. By use of a modified "Kleinschmidt technique", the pattern of chromosomes in unirradiated cells can be compared with that of irradiated cells in the electron microscope. Thus for the first time DNA double-strand breaks, chromosome aberrations and cell killing can be compared with one another in the same eukaryotic system and in the same dose range. The dependence of these cellular and molecular responses on radiation quality will be studied. As already explained, it is not possible using present day techniques to measure irreparable DNA double-strand breaks in mammalian cells in a biologically meaningful dose range. It is possible however, to measure the rate of repair of double-strand breaks and the influence on the rate of factors modifying cell survival after irradiation. A method has been developed for measuring double-strand breaks in Ehrlich ascites tumour cells utilizing the unwinding method of Ahnström and his colleagues. It may be possible to measure repair of double-strand breaks using this method not only in Ehrlich ascites cells but also in human cell lines under various conditions. The possibility of using liposomes to transport compounds, known to protect DNA in vitro but not normally able to penetrate the cell, across the cell membrane will also be investigated.

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Head(s) of research teams(s):

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Contract no.: BIO-E-420-81-B

General subject of the contract:
Genetic effects induced by radiations and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

Description of research work:

The heterozygosity for deleterious recessive mutations in human population may involve hundreds of genes and the somatic genetic risk of ionizing radiations may be principally due to the expression of these pre-existing mutations through aberrant mitotic segregations such as non-disjunctions and chromosomal breaks and rearrangements. Hereditary genetic load is caused principally by new mutations. Therefore this common programme proposes studies of both classes of these radiation-induced phenomena and in particular their prevention. When the genetic material of the cells of the body (somatic cells) or of the cells of reproductive organs (germ cells) is exposed to radiations or radiomimetic or carcinogenic chemicals, the following sequence of events ensues : after the lesions are produced, a signal is expressed ; this triggers various processes of repair and recombination (e.g. chromosomal rearrangements) involving diverse forms of DNA synthesis that will eventually restore a functional DNA structure with the original or modified nucleotide sequence. In bacteria the mutagenic effect of radiations and of chemical carcinogens is dependent upon the inducible error-prone DNA repair system. A similar pathway operates in mammalian cells and it may also involve an inducible system that generates chromosomal aberrations. All inducible phenomena are in principle preventable.

1. Study of radiation-induced DNA lesions. Homopolymer polydC:oligodG and viral ØX174 and SV40 DNA, irradiated with UV light or gammma-rays, will be used as substrates for DNA synthesis by purified bacterial and mammalian DNA polymerases and/or extracts from intact and irradiated cells. Lesions that block the elongation of new DNA chains as well as those that miscode directly for a non-complementary nucleotide will be identified. The former lesions belong to SOS inducing lesions, the latter lesions usually do not cause SOS induction, but only a site-specific mutagenesis. With our especially designed tester strain defined radiation-induced lesions will be introduced into bacterial and mammalian cells by infection or transfection to test their potency to trigger SOS induction, mutagenesis and chromosomal rearrangements.

2. Study of mechanisms of radiation-induced chromosomal rearrangements and of their prevention. Chromosomal phenotypes of the non-malignant cells from high cancer risk human mutants very much resemble chromosomal phenotypes of irradiated cells from normal individuals (chromatid exchanges and breaks). The work will be performed also with

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these human mutant cell lines in order to elucidate at least one rate-limiting step in carcinogenesis and its prevention. For testing the inducibility of radiation-provoked chromosomal rearrangements, cell fusion experiments between irradiated and non-irradiated partner cells with distinguishable chromosomes (e.g. BUdR labeling) will be done to determine whether DNA damage in one cell triggers chromosomal aberrations and sister chromatid exchanges among intact chromosomes of the fused non-irradiated partner.

3. Study of repair of radiation-induced damage. Purification and detailed analysis of known and new enzymes of DNA metabolism from human cells, such as DNA polymerases, DNA dependent ATP-ases, base excision enzymes, exo and endonucleases, DNA ligases, terminal deoxynucleotidyl transferase and DNA binding proteins, and their integration into multi-enzyme systems. Determination of their sensitivity to agents affecting cellular radiosensitivity, replication and repair. Isolation of mutant lines resistant to radiosensitizing agents. Reconstruction of mammalian DNA repair processes in subcellular systems : i.e. permeabilized cells, isolated nuclei and isolated metaphase chromosomes as substrates for in vitro multi-enzyme repair.

4. Study of fidelity factors of the replicational machinery that determine replicability mutagenicity of radiation-induced lesions. Mutagenic inducible lesion bypass DNA synthesis will be studied in ØX174 and M13 phage DNA in E.coli and also in a SV40/monkey cell system. Physiological factors affecting this mutagenic radiation-induced event will be studied in view of finding specific inhibitors of radiation mutagenesis.

5. Study of SOS induction - E.coli: Chimeric λ phages carrying cloned fragments of the replication origin of plasmid and cellular genomes can become SOS signal following irradiation. This suggests that possibly both DNA structure and functions determine its capacity to trigger SOS induction when damaged by radiation. - Mammalian cells : The capacity of damaged viral DNA and of specific DNA restriction fragments to induce provirus and/or mutagenesis will be studied in transfection and infection routes of DNA transfer into intact and irradiated cells.

6. Study of molecular nature of radiation-induced mutations. The precise nature of mutations induced by UV and ionizing radiations will be determined by extensive nucleotide sequencing of revertants and forward mutants in bacterial (M13) and mammalian (SV40) viruses. The role of DNA lesion as SOS induction trigger and mutation target will be determined by sequencing viral mutants that arose by the irradiation of either host cell alone, virus alone or both together.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-E-408-81-NL

General subject of the contract:

The genetic control and enzymology of DNA repair of radiation damage in prokaryotes and eukaryotes.

Description of research work:

The first project treats of the mechanism of the excision-repair process in Escherichia coli and its regulation.
In order to obtain an accurate, and more complete view of DNA repair processes, it must be considered imperative to clarify the interrelationships between the repair gene products, their mechanism of action and their regulation. The work to be initiated or continued by the contractant will involve the following research activities :

1. Molecular cloning of the uvrA gene; identification and characterization of the gene product.
2. A study on the regulation of expression of the uvrB gene by the UvrC protein using recombinant DNA techniques further refined in the laboratory.
3. An investigation on autogenic regulation of the uvrB gene for which preliminary evidence has been obtained. This work implies : 1) in vivo/in vitro construction of uvrB mutants defective in autogenic regulation and in vitro transcription of cloned uvrB DNA in the presence of purified UvrB protein.
4. DNA sequence analysis of the uvrB regulatory elements, in particular of uvrB mutants carrying altered regulatory elements. Similar studies can be envisioned with the cloned uvrA and uvrC genes.
5. In vitro transcription studies with purified components, i.e. uvr-containing recombinant plasmid DNA, RNA-polymerase and the Uvr proteins. Experimental lines involve: 1) transcriptional analysis using DNA-RNA hybridization techniques and 2) electron microscopic studies of the interaction of Uvr proteins with irradiated DNA and/or specific uvr regulatory DNA segments.
6. In vitro reconstitution of the 'incision enzyme' with the purified UvrA, UvrB and UvrC proteins in order to establish the function of each protein in the multisubunit complex.

The second project treats of the mechanism of mutagenesis, namely the role of radiation in the activation of transposable elements.
Bacteriophage Mu is a highly efficient, well characterized prokaryotic virus which behaves like a transposon. In fact, since transposition is an essential part of the viruses replication mechanism, Mu makes an ideal object for the study of transposable elements. The DNA carrying both the genes and the structures required for transposition have been cloned in the laboratory of Molecular Genetics, University of Leiden,

and a detailed investigation of the requirements for transpositional activity has been undertaken. Three elements appear to be involved in the movement of this transposon: 1) the physical ends of the transposon, 2) transposase, the product of gene A, and 3) the repressor gene which controls the expression of gene A. The work to be initiated or continued will involve the following research activities:

1. Investigation of the process of Mu integration and replication and the effect of radiation on these processes.
2. Maximalization of the expression of the Mu transposase gene and the purification and characterization of this gene product.
3. A study of the regulation of Mu integration.
4. A study of the G region of Mu as a model for a transposon with an important regulatory function.

The third project treats on mechanisms of DNA repair in human cells. In collaboration with 3 other laboratories a project is proposed with the purpose of studying repair processes in human cells. The laboratory of Molecular Genetics, University of Leiden, will contribute to this project by providing the expertise in recombinant DNA technology required for the cloning of human repair genes. Furthermore, the problem will be approached from the prokaryotic side by investigating the possible expression of E.coli repair genes in human cells and their ability to complement the defects of repair-deficient human cell lines. The following experiments will be undertaken.

1. Isolation of uvrA-, uvrB- and uvrC- mRNAs from E.coli.
2. A study of the expression of uvr-DNAs and uvr- mRNAs in an in vitro eukaryotic (wheat germ) transcription/translation system and in a semi in vitro system derived from Xenopus oocytes.
3. Modification of the regulatory regions of the repair genes by genetic manipulation allowing the genes to be maximally expressed in the eukaryotic systems.
4. Transformation of the manipulated prokaryotic repair genes into human Xeroderma pigmentosum (XP) cell lines, followed by complementation and repair studies of the 'transformants'.
5. In a later phase, the transfer of cloned human repair genes into E.coli followed by a comparative study of these genes with E.coli repair genes.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-E-427-81-F

General subject of the contract:
Genetic effects induced by radiation and chemical carcinogens : mechanisms common to DNA repair, replication, recombination and mutagenesis.

Description of research work:

The heterozygosity for deleterious recessive mutations in human population may involve hundreds of genes and the somatic genetic risk of ionizing radiations may be principally due to the expression of these pre-existing mutations through aberrant mitotic segregations such as non-disjunctions and chromosomal breaks and rearrangements. Hereditary genetic load is caused principally by new mutations. Therefore this common programme proposes studies of both classes of these radiation-induced phenomena and in particular their prevention. (See also C.N.R.S., Dr. Devoret, contract n° BIO-E-426-81-F, U.L.B., Dr. Radman, contract n° BIO-E-420-B, C.N.R., Dr. Falaschi, contract n° BIO-E-428-81-I). When the genetic material of the cells of the body (somatic cells) or of the cells of reproductive organs (germ cells) is exposed to radiations or radiomimetic or carcinogenic chemicals, the following sequence of events ensues : after the lesions are produced, a signal is expressed ; this triggers various processes of repair and recombination (e.g. chromosomal rearrangements) involving diverse forms of DNA synthesis that will eventually restore a functional DNA structure with the original or modified nucleotide sequence. In bacteria the mutagenic effect of radiations and of chemical carcinogens is dependent upon the inducible error-prone DNA repair system. A similar pathway operates in mammalian cells and it may also involve an inducible system that generates chromosomal aberrations. All inducible phenomena are in principle preventable. The general theme of this research programme is the study of relationship between the existence of DNA lesions induced by radiations or carcinogenic chemicals and the initiation of carcinogenesis. The DNA repair processes are very important in that orientation since a mutation event could either come from an unrepaired lesion or a lesion repaired by an error-prone process. This latter process, called SOS repair in bacteria will be studied in eukaryotic cells. Animal DNA viruses can be used as biological probe for studying SOS repair processes in eukaryotes. Cells are first treated by carcinogen then infected with UV-irradiated virus. The way irradiated virus is repaired in treated cells can tell us how SOS repair works. Such experiments will be carried out with monkey kidney cells and simian virus 40 (SV40). Numerous mutants of SV40 being well characterized, the mutagenic properties of SOS repair can be studied with this virus. For example, if infection of treated cells is carried out using thermosensitive SV40 mutants, the measure of

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reversion frequencies can be determined by scoring the number of plaques growing at 41° over the number of plaques growing at 33°. Moreover, the complete nucleotide sequence of SV40 genome is known and by sequencing various parts of SV40 revertants interesting results will be obtained on the molecular mechanism of the SOS repair mutagenesis. Preliminary experiments will be carried out by using human cells in culture infected with UV-irradiated SV40 mutants to determine whether or not such SOS functions could be detected in humans. In bacteria, it has been shown that SOS repair is induced in treated cells with other cellular processes called SOS functions. It is of interest to determine if such SOS functions are also induced in eukaryotic cells treated by carcinogens. Among the possible candidates to these SOS functions, are the induction of integrated viruses, the sister chromatid exchanges (S.C.E.) and the gentic recombination. The inducible properties of S.C.E. will be studied either by the technique of fractionated doses or by cellular fusion. It is proposed to irradiate Chinese hamster cells and to fuse them with other unirradiated cells in which S.C.E. will be measured. Finally, it will be interesting to find a good test to follow genetic recombination in animal cells. The use of DNA viruses can be useful for such a goal. For example, by infecting two different mutants of SV40 in the same cell, genetic recombination can be studied and quantified. The induction of SOS functions in such an infected cell could be correlated with variations in genetic recombination ability.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Dr. A. G. Searle
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Contract no.: BIO-E-429-81-UK

General subject of the contract:

Development of in vivo mouse models for human X-ray sensitive diseases.

Description of research work:

It will be attempted to develop an in vivo mouse model for X-ray sensitive human diseases such as Ataxia telangiectasia, Fanconi's anaemia, Huntington chorea and some retinoblastomas. Most of these diseases are characterized not only by an enhanced sensitivity to the induction of chromosomal aberrations by X-rays, but also by many congenital neuroanatomical and/or physiological abnormalities. Among the hundreds of mouse mutants generated during the last decades there are several with congenital abnormalities similar to the ones observed in human radiosensitive patients and these mouse mutants will be selected for initial screening for radiosensitivity. The screening of the selected mutants will be performed with the bone marrow micronucleus test using exposures of 50 and 100 rad X-rays and sampling times of 18 and 27 hours. For each dose and sampling time it is planned to use two males and two females. This procedure will generate quick and reliable information about the chromosomal radiosensitivity of mutant versus normal laboratory mice. After initial screening and identification of some interesting mutants more detailed genetic and biochemical analyses will follow. With respect to genetic tests, chromosome breakage by metaphase analysis and study of sister chromatid exchanges in (a) bone marrow cells, (b) peripheral blood lymphocytes, and (c) spermatogonial cells, is planned, together with the study of sister chromatid exchanges, chromosome breakage and point mutations in cell cultures derived from the mutant mice and normal laboratory mice. Other tests that can be of interest are dominant lethals and translocation induction in stemcell spermatogonia. With biochemical techniques such as neutral and alkaline sucrose gradients and neutral and alkaline elution DNA repair processes of radiation induced single and double strand breaks can be studied. Both sparsely and densely ionizing radiations will be employed. The study of heterozygotes, in case of autosomal linked mutants, will also be considered.
The choice of mutants at first instance will be brindled (Mo^{br}), fidget (fi), gyro (Gy) jimpy (jp), ochre $(Och)_{vbr}$, sprawling (Swl), varitint-waddler (Va) and viable-brindled (Mo^{vbr}). Other possible candidates are ataxia (ax), nervous (nr), staggerer (sg) and weaver (wv) which can be studied at later stages. Of this collaborative

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research effort the MRC Radiobiological Unit at Harwell will do the breeding of the mutants and part of the genetic tests whereas the screening, the biochemical analysis and part of the genetic tests will be performed in the Department of Radiation Genetics and Chemical Mutagenesis in Leiden (see contract n° BIO-E-421-81-NL).

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Head(s) of research teams(s):

Contract no.: BIO-E-407-81-NL

Dr. J.W.I.M. Simons
Dep. Radiation Genetics
and Chemical Mutagenesis
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General subject of the contract:

The genetic and biochemical basis of radiation sensitivity in human and other cells in culture.

Description of research work:

The project on genetic analysis of DNA repair aims to increase the number of radiation sensitive cell lines to be used for the study of DNA repair and to extend the methods which are available for their genetic analysis. To this end it is planned (1) to identify and collect cells from radiation sensitive syndromes as well as heterozygotes. Routine screening is carried out in cooperation with the University Hospital which will allow the identification of new repair deficient syndromes. In this way recently a new X-ray sensitive syndrome (Black Fan Diamond, a pure red cell anemia) has been identified; (2) to develop methods for the isolation of repair deficient mutants in cultured mammalian cells: (3) to develop a method for the study of mismatch mutagenesis, and (4) to develop an assay for the quantitative ascertainment of both cell transformation and mutation in one cell line. The second project will focus on biochemical analysis of DNA repair. It is possible now to detect and quantify several types of DNA damages produced by irradiation and chemical mutagens. Although for some lesions we partly understand how the cell tries to cope with them, it is still unclear at what point cellular processes are disturbed in such a way that genetic changes are generated. Work in our laboratory in which enzymes have been introduced into permeabilized cells showed that this can be a way to correlate the effects of these enzymes at the biochemical level with those at the genetic level. We will continue along this line and hope that this approach will tell us what lesions are important for mutation induction. Biochemical endpoints which will be analysed are single and double strand DNA breaks (following X-ray and neutron irradiation), cross links, pyrimidine dimers and alkylation adducts produced by chemical mutagens. We will use the technique of alkaline elution in order to improve the sensitivity of our measurements.
Another approach for the study of DNA repair will be the use of cell strains which have the capacity to photoreactivate pyrimidine dimers (Xenopus laevis, chicken and marsupial cells). An attempt will be made to transfer the gene for the photoreactivating enzyme from Xenopus cells to mammalian cells. We will study the effects on DNA-replication as well as on biological endpoints. We will continue with the use of UV-specific endonucleases in the analysis of DNA-replication following UV-irradiation. Preliminary experiments showed that we can visualize differences in DNA-replication between Xeroderma pigmentosum and wild

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type human fibroblasts. In the third project the consequences of DNA damage and repair will be studied via (1) the utilization of permeabilised cells to study the mechanism of induction of chromosome aberrations; (2) photoreactivation to assess the role of dimers in the production of SCE, chromosome aberrations, point mutations and cell killing ; (3) substitution of thymine by BUdR to study basic mechanisms of chromosome aberration formation; (4) liquid holding of cells treated with radiation or chemical mutagens in order to study the effect of excision repair on chromosomal aberrations, SCE's, mutations and cell transformation.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-E-413-81-UK

Dr. H. Smith
Biology Department
NRPB
Chilton, Didcot
GB-Oxon OX11 ORQ

General subject of the contract:

The production of chromosome aberrations in human lymphocytes by ionizing radiations.

Description of research work:

So far γrays, X-rays, neutrons of various energies and αparticles have been used to irradiate whole blood at 37°C with a view to measuring the aberration yields as a function of dose. All responses have been analysed in terms of the equation :

$$Y = \alpha D + \beta D^2 \qquad (1)$$

For the lower LET radiations γ-rays, X-rays, 7.6 and 14.7 MeV neutrons β is approximately constant at 6×10^{-6} rad^{-2}. For fission spectrum neutrons and alpha-particles, β is not significantly different from zero. This behaviour of β as a function of LET is consistent with the theory of Neary (1965). An analysis of the variation of α with neutron energy combined with our measurements using alpha-particles leads to the inference that at a low LET increases proportionally with LET, reaches a peak in the region of 70 keV/μ and thereafter falls rapidly with increasing LET. In order to investigate further this relationship track segment experiments using accelerated particles will be performed. Chromosome aberration yields are usually expressed as a function of dose using equation (1) where Y is the aberration yield, D is dose and α or β are fitted coefficients. For acute exposures the fit to the equation is generally very good but for chronic exposures the fit is poor. The D component, often interpreted as the number of aberrations caused by effects within the same particle track, is expected to be independent of dose rate. The term βD^2 is commonly interpreted as an interaction term between effects from two independent particle tracks and the magnitude of this term depends on the time interval between the two tracks. Thus the βD^2 is dose rate dependent. It was reported that the dicentric yields produced in lymphocytes exposed chronically to γ rays may be predicted from the relationship between dicentric yield and dose for acute exposures. For this conversion use was made of the G-function of Lea and Catchside (1942) and a mean repair time derived from fractionated exposures. The mean repair time is about 2 hours and good evidence for this value has been produced. It was concluded that the interaction coefficient for acute exposures should be reduced by a factor $\exp(-t_1/t_o)$ when fractionation effects are considered. Here t_1 is the time between fractions and t_o is the mean life (2 h) of active species. This idea has been extended to the statement that if t is the time for continuous protracted exposures

and $^{t}/_{t_o}$ is represented by x then β is modified by G(x) such that

$$G(x) = \frac{2}{x^2}\left[x - 1 + \exp(-x)\right] \qquad (2)$$

Thus yields for chronic exposures may be derived from the dose effect relationships for acute exposures using equation 3 where α and β are the fitted coefficients for acute exposures.

$$Y = \alpha D + \beta G(x) D^2 \qquad (3)$$

The modification G (x) is a function of irradiation time and requires the assumption made by Lea (1946) that the initial chromosome breaks decrease exponentially with time. Data from this laboratory suggest that long term breaks exist which remain available for recombination long after the majority of damaged sites have been rendered unreactive. This idea was originally discussed by Lea in 1946. The dose rate effect is usually examined experimentally by irradiating specimens to doses at a constant dose rate, i.e. by altering the length of time of exposure. In order to investigate properly the effect of the G-function modification on the α and β yield coefficients for continuous exposures it is necessary to construct a dose response curve at a constant exposure time, i.e. by varying the distances between source and samples. It is proposed to do this for γ radiation and to compare the curves obtained after irradiation for different times.

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Head(s) of research teams(s):

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Contract no.: BIO-E-406-81-NL

General subject of the contract:

The genetic effects of radiation in eukaryotes.

Description of research work:

The major goal of the research programme will be the collection of data on the genetic effects of radiation in eukaryotes. The investigations are specifically designed (1) to study the nature of radiation-induced genetic damage, the mechanisms involved in the production of mutations and chromosome aberrations and the processes of repair associated with these and (2) to obtain quantitative data on dose-effect relationships and on the modifying effects of different biological and physical variables on radiation-induced genetic damage. The studies fall into three groups, depending on the systems and methods used: (1)Drosophila (germ cells, genetic methods): (2) mammals, including primates (germ cells; cytogenetic methods) and mammalian (including human) somatic cells (cytogenetic and molecular methods) and (3) mammalian (including human) somatic cells (genetic and molecular methods).

1.Drosophila. Genetic and molecular studies of both prokaryotic and lower eukaryotic organisms over the last decade have led to substantial progress in our understanding of the role of DNA repair processes in the realization of genetic damage induced by mutagens and have catalyzed similar studies with other organisms. In Drosophila, the isolation and characterization of strains that are sensitive to specific mutagens (mus strains) and of those that have abnormal meiotic behaviour (mei strains) during the last 4-5 years, represent an important forward step: they enable studies on the genetic control of DNA repair and mutagenesis at a level that has not been hitherto possible. The main thrust of the Drosophila research effort will be towards gaining insights into the role of these repair processes in mutagenesis in male and female germ cell stages. The second aspect of the work concerns mutator genes; these have been found at high frequencies in natural populations of Drosophila and in species of organisms investigated in this respect. Neither their role in the population nor their effects on spontaneous and induced mutation is fully understood. The on-going work with a mutator strain (MR: in this a II chromosome mutator gene causes a significant increase in spontaneous mutation frequencies and also induces crossing-over in males) with respect to (1) the pattern of distribution of X-linked recessive lethals (spontaneous and X-ray-induced ones) in the MR strain; (2) the nature of mutations induced by MR (specifically, whether MR-induced mutations involve insertion of repetitive sequences (3) possible relationships between preferential sites of crossing-over and loci of high mutability and (4)

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the effects of MR in combination with repair-deficient mutations will be continued. The third aspect relates to neutron effects. Attempts to find a qualitative difference between the genetic effects of neutrons and X-rays have shown no differences for the homozygous viability of induced translocations and loss of sex-chromosomes. A significant difference between neutrons and X-rays was reported when mutation induction at three visible loci was studied. This work will be repeated to examine whether these loci are specifically affected in a different way by neutrons and X-rays. Fourthly, attention will be focused on a continuation of the work on mechanisms of induced genetic damage in meiotic and post-meiotic germ cells with X-rays and neutrons in addition to studies on the repair of damage by radiations of different LET in cells such as spermatids and mature sperm which have strikingly different nuclear volumes. Finally, the project on radiation-induced non-disjunction which is in progress, will be continued. 2. Mammalian cytogenetics. The programme is aimed at comparing cytogenetic damage induced by ionizing radiations in somatic and germ cells of mammals (mouse, rat, rhesus monkey, marmoset and man). Both structural (e.g., reciprocal translocations) and numerical (e.g., aneuploids produced by non-disjunction) chromosomal aberrations will be studied. The role of factors influencing the radiation- induction of chromosome aberrations (such as LET, dose-fractionation, radiation atmosphere, hormones etc.) will be studied both in vivo and in vitro. Another line of inquiry concerns the relationship between sister chromatid exchanges and chromosomal aberrations under different conditions of irradiation. Parallel biochemical studies on the frequencies of induced DNA lesions (double-strand breaks, single-strand breaks, base damage) will be made. It is also proposed to explore the possible utility of the technique of detection of thioguanine-resistant mutants (HG-PRT$^-$) in human peripheral blood lymphocytes for monitoring human populations for exposure to ionizing radiation. A further line of investigation is related to neutron induced lesions; the probable differences in the fate of X-ray and neutron-induced lesions in hepatocytes of rats will be studied immediately and after various time intervals following in vivo irradiation, using the frequencies of micronuclei in hepatocytes as indicators of chromosomal damage.

3. Mammalian cell genetics. Attention will be focused on (1) a further development and improvement of mutational assay systems in mammalian cells and (2) discrimination between point mutations and deletions in mammalian cells. Work on the first project aims to increase our knowledge on mutation induction in mammalian cells by X-irradiation. This pertains to (1) the number of markers to be used in assay systems; (2) conditions which may influence the effect of X-irradiation such as the comparison of mutation induction in vitro and in vivo or the comparison of anoxic conditions with conditions in air and (3) the number of genetic end-points e.g., forward mutation, reverse mutation, cell transformation and reactivation of the inactive X-chromosomes. The second project aims to determine the ratio of deletions to base changes induced by irradiation in populations of cells and to compare the fitness of populations differing in these ratios.

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Head(s) of research teams(s):

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Contract no.: BIO-E-396-81-D

General subject of the contract:

Isolation of the Zea Mays transposable controlling element.

Description of research work:

The isolation of the Zea Mays transposable controlling element will be attempted via the following steps: Mutants containing Ds in the vicinity of gene Sh on the short arm of chromosome 9 have been obtained from B. McClintock and homozygotes of them have been obtained. mRNA has been obtained from wildtype maize, and has been translated in cell-free systems for protein synthesis (rabbit reticulocyte and wheat germ). The synthesis of sucrose synthetase has been demonstrated by immunoprecipitation with an antiserum raised against purified sucrose synthetase, the product of gene Sh. mRNA has been reverse transcribed into cDNA, the latter has been cloned by elongation with dC and annealing to a PstI-cleaved, dG-elongated pBR322, and a number of cDNA inserts have been obtained. The cDNA inserts are presently tested for hybridization against sucrose synthetase mRNA by hybrid-selected translation. These studies will be continued until an appropriate cDNA clone is obtained. The cDNA clone will be characterized with regard to length, and will be used to enrich sucrose synthetase mRNA and to obtain more and longer inserts, if the first insert will be small. cDNA will be nick-translated and used as a hybridization probe to detect DNA fragments containing the sucrose synthetase gene (or parts of it) in total or partial restriction digests of nuclear DNA both of the wildtype and of the mutant. The sucrose synthetase gene and as much adjacent DNA as possible will be cloned in a suitable lambda vector (or cosmid) both from the wildtype and the mutant. Any DNA not present in the wildtype will be considered as candidate for Ds. This DNA will be tested for Ds properties by hybridization against the DNA of maize lines either containing or not containing Ds. The presence of silent copies will be looked for. Homology between Ds and its sister elements Ac will be tested. Ds will be identified (1) by obtaining no hybridization in Ds-free lines and positive hybridization in Ds-containing lines, or (2) by obtaining one additional fragment in Ds-containing lines, if there is already some hybridizing DNA in Ds-free lines, or (3) by hybridization against other clones, either obtained by us at the Adh locus, or by other laboratories. In case of success, physiological studies concerning the transcription and translation of Ds will be initiated.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-E-399-81-I

Prof. R. Strom
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General subject of the contract:

Repair of DNA in human and animal cells.

Description of research work:

The interaction between methylation of cellular DNA and repair will be investigated using cultivated human cells. Whereas previous research has concerned how cells strive to reconstitute base sequences in DNA in mechanisms among whose chemical principles are base-pairing and the properties of DNA polymerases, whilst current research indicates that in given tissues specific patterns of methylation of cytosine bases of DNA constitute essential and inherited biological signals additional to the base sequences, the research will seek to establish the existence and eventual nature of any systems in human cells that reconstitute the pattern of bases either directly damaged by radiation (e.g. in formation of pyrimidine dimers by UV radiation) or removed in the course of repair and replaced by nonmethylated bases. Since many cells methylate only the newly synthesised DNA strand and not the "parental" strand present before the preceeding S phase, an early object of the research will be to discover whether they methylate repair patches in the parental strand in the G phases. Eventual objects of the research will be to discover evidence about whether there exist separate mechanisms for normal and repair methylation analogous to the separate mechanisms for normal and repair DNA synthesis and if, when DNA methylation is interfered with, this increases the radiosensitivity of the cells, and the relation of methylation with cytosine deamination as a mutagenic mechanism. The material used will be human cells in culture and nuclei extracted from them.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. Dr. J. Sybenga
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Contract no.: BIO-E-477-81-NL

General subject of the contract:

Genetic background damage accompanying fast neutron and X-ray induced chromosomal aberrations.

Description of research work:

Fertile M_1 plants of rye, exposed as seed to fast neutrons or X-rays, have been isolated for the production of a M_2 generation.
The M_2 generation consists of 297, 349 and 128 M_2's respectively for the neutron irradiations and 267, 399 and 198 M_2's for the X-ray irradiations. The genetic damage accompanying the translocations will be assayed by scoring the frequency of translocation homozygotes in segregating progenies in relation to overall fertility. All levels of damage between lethality and almost-normal can thus be recovered.
All viable M_2 plants will be analysed cytologically for somatically recognizable translocations, and subsequently checked in meiosis. Heterozygous translocation carriers will each receive three treatments:

a. selfing
b. backcrossing to the parental inbred line
c. outcrossing to two unrelated inbred lines.

Ad a.: Selfing of heterozygotes yields data on homozygote viability (of the translocation and the normal chromosome) in M_2 and subsequent generations and as much fixation of sublethal damage as tolerated by the plants.
Ad b.: Repeated backcrossing followed by selfing in subsequent generations separates background from breakpoint damage for the same translocation as in a.
Ad c.: Outcrossing places these translocations in a different genetic background, with possibly very different homozygote expression.
In all three treatments several generations are required. Since each generation takes about six months and in view of the laborious cytological screening, three years at least are required to complete the programme, and probably four. Preliminary data are expected after two years.

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Head(s) of research teams(s):

Prof. Dr. A.J. van der Eb
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Contract no.: BIO-E-405-81-NL

General subject of the contract:

Molecular biology of the repair of DNA damage in mammalian cells, and its relationship with mutagenesis and oncogenic transformation.

Description of research work:

1. Studies on the possible relationship between repair of DNA damage, mutagenesis and oncogenic transformation in animal cells.
Treatment of cells with low doses of X-rays or UV triggers processes enhancing the survival of radiation-damaged infecting viruses. It was shown that this induced reactivation of survival is correlated with an increased incidence of mutations. The purpose of this research project is to further study in mammalian cells the relationship between induced reactivation of survival and mutagenesis on one hand, and the sensitivity to transformation by DNA tumor viruses (or other agents) on the other hand. In addition we plan to study whether such error-prone reactivation processes are correlated with induction (activation) of integrated viral DNA from transformed cells.
Optimal conditions for expression of X-ray or UV-induced reactivation of UV-irradiated MVM or H1 parvoviruses will be determined in hamster or rat cells, irradiated with low doses of X-rays or UV (direct Weigle-reactivation"). It will be investigated whether optimal expression of reactivation is correlated with an increased sensitivity to transformation by viruses (e.g. SV40) or other agents. It will also be tested whether such conditions favor activation (induction) of integrated viral DNA in transformed cells, e.g. in SV40-transformed cells or of endogenous C-type viruses in murine cells. The hypothesis will also be tested that viral transformation genes may have pleiotropic functions and that one of their activities involves induction of error-prone repair mechanisms.
To further investigate the mechanism of induction of error-prone repair it will be tested whether these processes can also be activated by introducing UV-damaged DNA into the cells rather than by irradiating the cells themselves. UV-irradiated SV40 or UV-irradiated mammalian DNA will be used as the potential induction signals, and SV40 or parvovirus as the probe(s).
2. Studies on the relationship between repair of DNA damage, mutagenesis and oncogenic transformation in normal and repair-deficient human cells. Several genetic diseases are known in man, which are characterized by defective repair of DNA damage. Such patients not only exhibit a high sensitivity to radiation or other DNA damaging agents, but they often also show a high incidence of cancer. This suggests that there will exist a relationship between (defective) repair and carcinogenesis. The purpose is to study some aspects of this

relationship in repair-deficient human cells. It will be tested whether normal human cells contain X-ray induced repair mechanisms of DNA damage, using X- or UV-irradiated SV40 or parvovirus as probes, and whether these activities are error-prone. Similar studies will be carried out with radiation-sensitive human cells. It will be investigated whether the sensitivity to transformation by SV40 (or other agents) is higher in radiation-sensitive cells than in normal cells and whether this can be influenced by introducing DNA damage.

3. Characterization of the nature of radiation-induced mutation. Virtually nothing is known about the nature of the mutations induced in mammalian cells by radiation (or repair processes). The purpose of this project is to obtain information on the molecular nature of such mutations by DNA sequence analysis. Revertants of SV40 ts mutants, that have been isolated during the former contract period will be used for DNA sequence analysis. The approximate positions of the secondary mutations will have to be determined by marker-rescue experiments. Attempts will be made to investigate whether the mutations induced in UV-irradiated virus have been caused by a "targeted" or an "untargeted" mechanism

4. Identification of repair genes using recombinant DNA technology. Patients with genetic defects of repair mechanisms of DNA damage are characterized not only by a hypersensitivity to DNA damaging agents but also by a high incidence of cancer. The molecular and genetic basis of the relationship between (defective) repair and carcinogenesis is unknown. The purpose of this project is the identification of the genes involved in repair of DNA damage in human cells. We anticipiate that this will eventually lead to a better understanding of repair-related carcinogenesis. Recently techniques have become available for the introduction of foreign genes into mammalian cells. These methods are based on the "Calcium technique" which was developed in this laboratory in 1973. Recent developments of the DNA transfection technique allow the selection of cells showing a high capacity of incorporation of foreign DNA. 1) It will be tried to transform radiation sensitive cells (Xeroderma pigmentosum, XP, will be used initially) to radiation-resistant cells, using DNA isolated from the following sources : (a) fragmented DNA of normal human cells, (b) fragmented DNA of herpes simplex virus (HSV) or vaccinia virus. These two viruses are chosen since both HSV1 and vaccinia virus have large genomes and are known to be less sensitive to UV, when tested on XP cells, than expected. This suggests that these viruses might code for their own repair enzymes. 2) If a (partial) correction of the repair defect is obtained in certain XP cells, attempts will be made to identify and clone the complementing cellular or viral genes using recombinant DNA techniques.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Prof. Dr. A.J. van der Eb
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NL-2333 AL Leiden

Contract no.: BIO-E-476-81-NL

General subject of the contract:

Molecular biology of the repair of DNA damage in mammalian cells, and its relationship with mutagenesis and oncogenic transformation.

Description of research work:

The contractant will extend his studies with xeroderma pigmentosum (XP) to other genetic syndromes in man which are characterized by a sensitivity to DNA damaging agents. The project will be carried out in collaboration with Prof. Bootsma, Rotterdam.
The following studies will be carried out :

1. Host-cell reactivation (HCR) of SV40 or SV40 DNA, treated with various doses of a DNA damaging agent, will be tested in diploid cultures of the following genetic syndromes : Ataxia telangiectasia (SV40 treated with X-rays); Fanconi's anemia (SV40 treated with DNA cross-linking agents); Gardners' syndrome (SV40 treated with UV); Cockayne's syndrome (SV40 treated with UV); xeroderma pigmentosum variant (SV40 treated with UV); other syndromes when they become available. The purpose of this study is to investigate whether the putative repair defects of these syndromes can be identified by means of the host-cell reactivation method using SV40 as a probe. The host-cell reactivation studies with Fanconi cells have already been started in collaboration with Dr. F. Arwert, Amsterdam.
2. If a repair defect can be identified in one or more of the genetic syndromes by HCR of damaged SV40, this approach will be used to study genetic heterogeneity by : (a) determining HCR in different representative cell strains of the syndrome involved; (b) performing complementation analysis by studying HCR of SV40 in heterokaryons of different representatives of the syndrome, in collaboration with the laboratory of Prof. Bootsma, Rotterdam.
3. HCR experiments as described in (1) will also be carried out with vaccinia virus or herpes simplex virus type 1 (HSV-1). If the survival of vaccinia virus or HSV-1 treated with DNA damaging agents is the same in cells of the genetic syndrome as in normal cells, this could indicate that the viral genome is independent on host-cell functions for the repair of its DNA, and that it might contain genetic information capable of repairing the damage in its DNA. This study might eventually lead to a new research project aimed at the identification and cloning of the active repair gene of the virus. - These experiments will be carried out only if a clear HCR effect is found with SV40 as the probe.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Radiobiology Unit
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GB-Oxon OX11 ORD

Contract no.: BIO-E-453-81-UK

General subject of the contract:

Factors affecting the yield of mutations from spermatogonial stem cells of mammals.

Description of research work:

Reciprocal translocations and specific locus mutations can readily be recovered from mouse spermatogonial stem cells following X-ray exposure. For both genetic end-points the dose-response curve with single exposures in bell-shaped, yields initially increasing with dose but then declining to lower levels after higher doses. However, when exposures are given in two equal or near-equal fractions 24h apart a linear dose-response consistent with additivity is obtained for translocations and an augmented response for specific locus mutations. Further fractionation experiments with X-rays have shown that the high additive translocation response obtained at higher doses after 24h fractionation is maintained with a 48h interval between fractions. However, with longer fractionation intervals translocation yields were sub-additive and remained so until (as judged by fertility data) spermatogonia surviving the first exposure had repopulated their numbers sufficiently that spermatogenesis was again proceeding by the time the second exposure was given. At this point, additive yields were again obtained. In a further series of experiments employing unequal sized X-ray fractions high translocation yields were obtained with high X-ray doses when these were preceded 24h earlier by a small conditioning dose. The varying responses obtained with different fractionation regimes have been interpreted in terms of a "triggering" by the first dose of the normally slowly dividing stem cells into a more active cycle to achieve repopulation following the depletion in their numbers through cell killing : In the first 24-48h, surviving stem cells may be "synchronized" into a radio-sensitive state prior to entering a shorter cell cycle : over the next few days most cells may proliferate rapidly, when they are highly sensitive to killing, whereas other may re-establish the normal slow cell-cycle and be more resistant to damage; finally, on re-establishing a near-normal population size, normal stem cell kinetics may resume and a "normal" radio-response is regained.

Consistent with the above interpretation is the observation that sub-normal translocation and specific locus mutation yields are obtained from the rapidly-proliferating stem cell populations of the immature testis. More striking, however, is the recent finding that a pre-treatment with TEM, which kills stem cells but does not induce recoverable translocations in such cells, modifies the subsequent genetic response to X-rays in apparently the same way as a conditioning

dose of X-rays. The modified response of damaged populations is therefore a general one not limited to X-rays.
An incidental discovery in these experiments was that TEM treatment given several days after X-ray exposure may actually reduce the X-ray translocation yield.
The above findings indicate that interactions with predictable genetic consequences will occur following exposures of spermatogonial stem cells to agents (chemical or physical) which cause stem cell killing and/or genetic damage. It is proposed to amplify and extend these findings for both chromosome breakage and point mutation events in the mouse and, since the purpose of this work is to assess the genetic risk to man of exposure to ionizing radiation or environmental mutagens and the consequences of interactions between such exposures, complementary studies in species other than the mouse will ultimately be undertaken. It may be expected that the genetic responses will vary according to the repopulation dynamics of the stem cell in each species.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-E-457-81-UK

General subject of the contract:

Non-disjunction studies with Robertsonian translocations in mice.

Description of research work:

Non-disjunctional events contribute significantly to the genetic load in man but the extent to which environmental radiation or other environmental agents influence the observed frequency is not yet well understood. Techniques for measuring non-disjunction are available in non-mammalian species but, although various assay systems have been developed in mammals, all have so far proved to have various limitations. Non-disjunction will be studied in mice with a normal complement of acrocentric chromosomes, and in those carrying Robertsonian translocations giving increased frequency of non-disjunction. The studies will be aimed at developing improved methods for study of non-disjunction, and investigating the effects of radiation on its frequency, and elucidating the mechanisms involved.

Most Robertsonian translocations (metacentrics) of feral mouse origin cause high rates of non-disjunction when heterozygous with the homologous acrocentric chromosome of the house mouse. Non-disjunction has been scored by assaying foetal loss (due to monosomy and trisomy), by metaphase II counts in spermatocytes or oocytes, and by intercrossing heterozygotes, one parent of which has both acrocentric and homologous metacentric arms marked with a suitable recessive gene. The latter system screens for complementary non-disjunction in the two parents which leads to the appearance of marked progeny in the F1. The marker method with two Robertsonian translocations of tobacco mouse origin has been used but it remains to be established whether the non-disjunction associated with these metacentrics is increased by ionizing radiation or other mutagens. Preliminary studies with other metacentrics of tobacco mouse origin have been carried out using this method, which proved practical for all chromosomes tested.

In recent years each of the tobacco mouse metacentrics have been established on a house mouse genetic background and gene markers have been introduced onto one or both arms of many of them. In the process, investigations have shown that at least some of the metacentrics when heterozygous suppress crossing over proximally in the arms involved and many display irregular segregation in one or both sexes. It is not known whether these other properties of the metacentrics are associated with the non-disjunction events. Several Robertsonian translocations of spontaneous origin have also been detected in house mouse stocks. These, too, have been established in separate stocks and markers are being introduced onto their chromosome arms. The proposal is to

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Continuation contract no.: BIO-E-457-81-UK

investigate further the various properties of the Robertsonian translocations with respect to non-disjunction. The effect of the transfer to laboratory mouse genetic background on the non-disjunctional properties of the various wild-derived metacentrics will be studied. The possibility that the non-disjunction frequencies associated with each metacentric can be increased by radiation or other mutagens will be tested. Multiple metacentric stocks with appropriate markers for use with the marker method will be developed, so as to screen with a number of chromosomes at once and hence increase the efficiency of the method. The possibility of using tester mice in the marker system carrying metacentrics having monobrachial homologies will also be investigated, so possibly increasing the recovered non-disjunction rates and the efficiency of the method even further.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-E-412-81-UK

General subject of the contract:

The radiobiology of cultured mammalian cells.

Description of research work:

The programme is divided into 3 projects : these broadly cover the biophysical aspects of dose-response relationships for a variety of radiobiological endpoints, quantitative and qualitative studies of radiation mutagenesis, and the role of post-irradiation repair functions in the response of irradiated cells.
Mammalian cells, including human and hamster, will be used to study the induction of chromosome aberrations, mutations and cell after inactivation. The existing data on relative biological effectiveness (RBE) of different radiation qualities (especially ultrasoft X-rays and slow atomic ions) will be extended to allow further comparison of the dose effect relationships of the different biological effects and of the relative dependence of RBE and radiation quality parameters such as track length and ionization density. Interpretation of these relationships will be made in terms of the energy and spatial requirements of the primary physical lesions which lead to the biological effects. This will involve further analysis of the track structure and microdosimetric properties at the nanometer level of ultrasoft X-rays, low-energy electrons and heavy ions. This biological and physical information will be used to constrain the hypotheses and predictions of a variety of alternative models of radiation action based on dose-effect and RBE-quality relationships. Additional constraints may be imposed by studies of the modification of the observed biological effects by various physical and biological factors and by radiation studies of mutant cells with alterations in radiation sensitivity. Well-characterized mutation systems (particularly for autosomal gene mutation) are needed to gain a representative estimate of the response of the mammalian genome to ionizing radiations. Refinement of existing mutation systems and the development of further new systems (including ones using single cells rather than colony growth to detect mutation) will be continued. Mutants will continue to be screened for biochemical and cytogenetic changes, as well as assessing their phenotypes in other ways (such as ability to complement in cell hybrids). An attempt will be made to establish whether the mutants with chromosomal re-arrangements have suffered loss of chromosome material (through incomplete exchanges) or have persistent gene expression changes (position effects). It is anticipated that these studies will also lead to the recognition of new "marker" strains

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for use in a variety of genetic experiments. The use of new genetic methods (selection of cell hybrids containing specific chromosomes, transfer of genes between cells, use of restriction enzymes to cut DNA, isolation of specific sequences using reassociation techniques, characterization of isolated DNA by biochemical techniques) to characterize DNA from mutants will be made during this period. The role of DNA repair functions in the mediation of radiation-induced cellular changes is well-established in microbes, largely as a result of studies with repair-deficient mutants. Whilst cellular repair is frequently invoked to explain dose-response relationships for the induction of various radiobiological endpoints in cultured mammalian cells, critical evidence on the nature and relevance of this repair is lacking. The availability of radiosensitive (provisionally repair-deficient) strains now provides the opportunity for detailed "repair" studies with cultured cells. Studies have been made of the basic radiobiology of radiosensitive ataxia telangiectasia (AT) human fibroblast strains with particular reference to their deficiency in the repair of potentially-lethal radiation damage and their relative sensitivity to radiations of different qualities. Because the interpretation of such data is dependent upon the characterization of the genetic and biochemical defect(s) in such radiosensitive strains it is proposed that these studies are extended in the following ways : (1) Investigate genetic complementation amongst AT and other cell strains using cell fusion and co-cultivation techniques; (2) Characterize chromatin and chromatin-associated proteins in normal and radiosensitive cell strains; (3) Investigate the activity of various DNA-related enzymes in extracts of normal and radiosensitive cell strains; (4) Isolate and characterize radiosensitive mutants produced de novo from established cultures of human and hamster cell lines; and (5) Genetic manipulation of repair capacity by gene transfer techniques.

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Head(s) of research teams(s):

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Contract no.: BIO-E-454-81-UK

General subject of the contract:

A study of the induction, transmission and consequences of chromosomal aberrations in the Syrian hamster (Mesocricetus auratus).

Description of research work:

The Syrian hamster, an organism widely used in the study of carcinogenesis, is somewhat unusual in its cytogenetics.

a) About 30 % of the karyotype consists of late-replicating chromatin ("Heterochromatin") and in contrast to most other mammals, the bulk of this is not located at or near the centromeres.

b) The animal can tolerate and transmit a considerable amount of chromosomal imbalance of this late-replicating material, including nullisomy, with almost no phenotypic effects.

From the cytological point of view, the animal is excellent material : the chromosomes band well and are readily distinguishable. Skin and blood grow well in culture, and the heterochromatin confers such distinctive band patterns during replication that the chronology of the DNA synthesis phase can be mapped in considerable detail.

The following lines of investigation will be pursued :

1) The transmission of unbalanced chromosomes with especial reference to the behaviour of cells during meiosis and foetal development.

2) The effect of imbalance upon radiosensitivity particularly with respect to differences between heterochromatin and euchromatin.

3) The induction by radiation and certain selected chemicals of chromosomal aberrations during S-phase, with especial reference to the role of the late-replicating heterochromatin.

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Head(s) of research teams(s):

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Contract no.: BIO-E-452-81-UK

General subject of the contract:

Development and use of methods for detection and analysis of deletions in the mouse, and of somatic mutations at the cellular level.

Description of research work:

The induction of deletions will be studied by the use of closely linked gene pairs (as has already been done with dilute and short-ear, d,se), since the apparent simultaneous mutation of both members of such a pair would indicate that a deletion had been induced. Mouse stocks are being developed involving the gene pairs : non-agouti and brachypodism (a and bp; chr. 2), dilute and short-ear (d and se; chr. 9), ruby and pale ears (ru and ep; chr. 19) and pink-eyed dilution and ruby-2 (p and ru-2; chr. 7). When the stocks are suitably developed the induction of deletions in germ cells will be tested by irradiating male or female animals at various gametogenic stages. It is anticipated that there may be wide variations in sensitivity of different stages to induction of deletions. Possibly deletions of the X-chromosome may also be studied, using X-linked markers. Methods for study of somatic mutation may prove valuable, in the use of the "parallelogram" method of extrapolation from one species to another. It may be of particular value if the same gene loci can be studied in somatic and in germinal cells. The methods to be developed in this proposal involve two gene loci, dilute (d) and pink-eyed dilution (p) used in the standard specific locus test for germinal mutations and cater separately for forward and reverse mutations. They depend on the fact that certain mutational effects can be detected in individual pigment cells (melanocytes) examined at an appropriate time with the result that a very large number of cells ($> 10^5$) can be screened in each treated individual. For forward mutations the dilute (d) and leaden (ln) loci are used since known recessive mutants at these loci cause gross morphological changes in the melanocytes. It is proposed to attempt to make the system suitable for automation, to explore dose-effect relationships, and to search for other suitable loci for this test. For the reverse mutation test, the pink-eyed dilution (p) locus may be used. In the retinal pigment epithelium (RPE) of animals homozygous for recessive alleles at this locus reversions of a single cell to wild-type can be detected. A system for measuring mutation rates by this means will be developed and validated using radiation and chemical mutagens.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-E-417-81-DK

General subject of the contract:

Chromosome pairing and disjunction in human meiosis.

Description of research work:

The assessment of the effects of radiations and radiomimetic agents on human male and female meiosis, i.e. on genetic recombination and chromosome disjunction requires a detailed knowledge of chromosome pairing and chiasma formation at the ultrastructural level. During the last four year period such knowledge has been obtained for the human spermatocytes. The interpretations have been supported by complementary studies on Bombyx and Coprinus.

The present programme comprises the following aspects :

1) The stages from late pachytene to prometaphase I in human spermatocytes will be analysed by three dimensional reconstruction in order to obtain a detailed knowledge how chiasmata are formed and stabilized. This is necessary for the definition of possible errors in these processes which may result in non-disjunction. Work presently in progress on males of Bombyx and Coprinus have shown that such a study is feasible and taught how the relevant and short stages of diplotene and diakinesis can be recognized in human spermatocytes.
2) A comparative study of the diplotene chiasmata in the human oocyte of primordial follicles in the ovary, a stage which may last as long as 50 years, will be undertaken.
3) An analysis of long term effects of radiation exposure on chromosome pairing and crossing over is carried out on biopsies from patients with testis cancer. Control biopsies (75 cases) prior to radiation treatment have been embedded for ultrastructural analyses. Whenever possible new biopsies after restoration of spermatogenesis are taken and will be analysed for damage and in relevant cases compared to the controls.
4) Chromosome pairing and recombination nodule distribution will be analysed in triploid and tetraploid Bombyx males as well as in allo-hexaploid wheat. This is undertaken to better characterize the effects of crossing over in homologous, non-homologous and homoeologous pairing. Such studies cannot be pursued in human material.
5) Short term effects of radiation on chromosome pairing and chiasma formation cannot be studied in human material and will therefore be analysed with Bombyx. Cultivation of male and female silkworms will be set up in collaboration with Dr. H. Akai, Sericultural

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Continuation contract no.: BIO-E-417-81-DK

Experiment Station, Tokyo. Irradiation of precisely defined meiotic stages will be carried out and the immediate effects analysed at the ultrastructural level. Specifically it will be investigated whether the zygotene stage with its naturally occurring efficient repair system for chromosome breaks will be more radiation tolerant than other meiotic stages.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-E-418-81-DK

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General subject of the contract:

Molecular events on the biologically active chromatin form of a single eukaryotic gene in response to radiation.

Description of research work:

The Tetrahymena system allows investigation of some of the molecular events which occur on the level of a single gene in response to radiation. Among the many questions which appear the following will be answered :

(I) Relationship between radiation damage caused in vivo and in vitro on a specific gene. In order to answer this question techniques will be used which make it possible to monitor and compare radiation damage on the chromatin by three different techniques : (i) The "snap-back" technique which takes advantage of the fact that the isolated chromatin is a giant palindrome. This technique is very sensitive in the range of 1-2 single strand breaks per gene. (ii) The transcription assay allows one to monitor various types of damages on the gene. The method takes advantage of the endogenous RNA polymerase activity on the chromatin and the different fidelity of the enzyme in the presence of different divalent cations. (iii) Finally, the exact position of a damage can be visualized in the electron microscope after binding of a specific protein. By a combination of these techniques it is possible to compare the relative radiosensitivity between (a) the gene in vivo, (b) the gene isolated in the biologically active chromatin form, and (c) the naked DNA. Based on these results it might be possible to make quantitative extrapolations of the radiation genetic hazard to man and to develop an in vitro assay system.

(II) Differences in the number of types of radiation damage made on the active versus the inactive part of the isolated chromatin. Nuclease digestion studies have demonstrated that the two regions of the gene have different structural organizations. The transcribed region contains a "loose" structure with little or no nucleosomal or higher structure, while the non-transcribed region (20 % of the total gene) has a condensed well-defined nucleosomal structure as well as elements of higher order structure. The system is an important tool in answering the question if active regions are more sensitive to damage than inactive regions. The question can be answered by the technique described in part (I).

(III) Differences in the rate at which the various types of damages are repaired in the active versus the inactive part of the gene.

The studies will be performed in vivo and might reflect the situation in man where different genes are expressed in the different organs. Thus, it might to some extent be possible to explain the differences in

radiosensitivity observed in different tissues. In this connection it is of greatest importance to ask if biologically active drugs interact differently with the lesions depending upon the chromatin structure. Preliminary studies have demonstrated that the chromatin both in vivo and in vitro can be sensitized up to 8 fold by various drugs.
(IV) Synthesis of specific monoclonal antibodies to proteins induced by electron irradiation of cells. Upon electron radiation, a DNA polymerase activity is induced up to 50 fold in the mitochondria of the irradiated Tetrahymena cells. The polymerase is encoded for by the nuclear genome. The DNA polymerase has been isolated and specific monoclonal antibodies will be raised. Such antibodies will enable us to localize the polymerase within the cell and study the function of the enzyme.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):
Commission of the European Communities DG XII-F
Biology, Radiation Protection and Medical Research
Biology Group Ispra
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Contract no.: CEC-DG XII-F-ISPRA

General subject of the contract:

Genetic and biochemical analyses of radiosensitivity and DNA repair.

Description of research work:

A) Genetic analysis of repair sensitivity

The general objectives and features of the programme are :

- to assess in complex eukaryotes the spectrum of the genetic effects induced by low doses and low dose rates which cannot be evaluated for the time being in mammalian systems because neither haploid cells nor regeneration systems are available for detecting and characterizing genetic changes.
- to take advantage of the unique properties of plant cells which can be handled in haploid conditions and regenerated afterwards in differentiated fertile mature plants for establishing a new and complete approach to the induction and genetic analysis of recessive DNA repair deficiencies in large populations of single cells and in the differentiated organisms which can be derived from them.
- to take advantage of the fact that plant cells can be induced at will into states of differentiation and de-differentiation for attempting to establish, for the first time in cultured cells of complex eukaryotes, an exact relationship between physiological phase, developmental stage, and radiosensitivity.
- to explore further the recently discovered features of cultured cells of Med-fly (presence of DNA-β polymerase, high density of initiation points of replication in the chromosome) for assessing the factors involved in the very high radioresistance of this species.

B) Biochemical mechanisms of DNA repair and sensitivity

The general objectives and features of the programme are :

- to contribute to the understanding of the complex pathways of DNA repair in human cells through the detailed characterization of several enzymes known to act on altered DNAs from mammalian and human tissues. In addition, efforts will be carried out to extend certain portions of this work to the eukaryotic organisms (plant cells and Med-fly cells) used as test material in the part of the programme dealing with genetic analysis.
- to prepare the ground for testing, at molecular level, the radiation sensitivity of lymphocytes from occupationally exposed individuals and of leukemic cells from patients undergoing radiotherapy. For this purpose, attempts will be made to correlate the inhibition by low doses of the formation of functional DNA replicons during the S-phase to the expression of genes coding for marker proteins that are specifically detectable in lymphoid cells by immunofluorescence

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methods.

- to analyse, in highly radiosensitive embryos (mouse), the effect caused by short-range β emitters at concentrations well below the maximum permissible body burden and incorporated or localized nearby the genetic material.
- to continue the distribution as a regular service to laboratories participating in the Community Programme of synthetic DNA-like substrates for assaying DNA enzymes.

1)

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-446-81-I

General subject of the contract:

Reduction of patient exposure preserving image quality in diagnostic radiology.

Description of research work:

Imaging systems of higher sensitivity (speed) reduce patient exposure in diagnostic radiology, but an increase in sensitivity can affect image quality.
The physical factors affecting exposure and image quality are complex and will be studied by application of the concepts of information theory.
The method quantifies the information content of the various imaging components so it is possible a trade-off between patient exposure and imaging quality.
It is believed that , on the average, patient radiation dose can be reduced approximately tenfoldby physical optimisation of the performance of diagnostic X-Ray systems. Therefore the physical optimisation of some diagnostic X-Ray systems will be studied, beginning from those of higher risk (for instance : mammography, CAT scan of the brain, etc..). The problem of breast examination differs from those of other diagnostic procedures for the scarce density difference of the soft-tissues structures and for the importance of detecting minute details as microcalcifications.
The image information content will be expressed in terms of object contrast, X-Ray beam quality, imaging system characteristics, and the image quality interrelationships with patient exposure will be considered.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-449-81-EIR

General subject of the contract:

Analysis of radiation doses to patients in diagnostic radiology.

Description of research work:

The research programme may be subdivided in four parts, each one answering a distinct objective :

(a) Estimation of the annual number of radiological examinations performed.

It is expected that the total number of examination of all types performed in a year would be about 800,000. In order to obtain detailed information about this, it is proposed that for certain periods during the year (e.g. 4 periods of a fortnight each) the following information would be sought in respect of every examination performed in the country : (1) the examination type. (2) the sex of the patient. (3) the age of the patient. It appears probable that this information could fairly easily be recorded in the individual X-ray departments, provided the co-operation of the staffs had been obtained and advance notice was given of the periods for which the information was required. The resources required to transfer this information from the individual departments are not easy to assess. It seems probable that in some cases the departments might be able to provide the information fairly easily. In other cases it woul be necessary for staff to visit the department and, in consultation with the department staff, obtain the information from the records kept during the analysis periods. It will also be necessary to consider how this mass of information is to be assembled and analysed.

(b) Measurement of the gonad doses and bone marrow doses delivered to patients.

For each type of examination it is proposed to make measurements of the radiation dose delivered to the patient for a number of different patients in different hospitals. The measurements would be made using either ionisation chambers or TLD dosemeters, or both. Measurements would be made of the dose delivered to accessible points on the patient so chosen that estimates of the gonad dose and bone marrow dose could be made from the available results of phantom measurements taken under comparable conditions. In order to reduce the standard error of the estimate of the average dose for that examination type, it will be necessary to carry out measurements on a number of patients. The same general procedure will be followed for each type of examination. It is expected that about 1,200 measurements would be performed during a year. These measurements would be distributed among the different types of examination in accordance

with the expected relative importance of the examination type. In order to get an unbiased representative result the measurements should be distributed over a number of different hospitals picked at random. When the best estimates of the average gonad dose for each examination type has been obtained in this way, it can be multiplied by the annual number of such examinations performed to calculate the contribution to the genetically significant dose, account taken of the distribution of the number of examinations between the sexes and among the age groups. The estimate of the bone marrow dose to the population would be carried out in a similar way.

(c) Inventory of the radiological equipment in use

Information could be sought from each institution concerning the number of X-ray units in use. For each unit the following details might be sought : manufacturer, type or model, year of installation, use : (Radiography, Fluoroscopy, Portable), method of field definition (e.g. light beam diaphragm, cones, other), image intensifier

(d) Assessment of the quality of radiological equipment

When a hospital is visited for the purpose of making measurements on patients a number of measurements would also be made on the X-ray units involved with particular reference to those features which would influence the dose to the patient. These would generally include the following : (1) H.V.L. at various KV settings; (2) Radiation output under selected conditions; (3) Relationship between field size on patient and light beam diaphragm or cone. In addition the following would be noted for each examination performed K.V, MAS, F.S.D., Type of screen used, use of gonad or other shield. If it was thought desirable further tests could be added to these, e.g. focal spot size (or image resolution), leakage radiation from tube housing, operation of automatic brightness controls, tests or processor function. It might be possible, by comparing the results from one hospital with those from another to gain some information about the connection between equipment condition and patient dose. But it should be noted that commonly the equipment will not be the only factor which will vary and it might well be difficult to attribute any observed differences to the equipment alone.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-373-81-I

General subject of the contract:

Radiation protection in medical diagnostic procedures.

Description of research work:

The studies carried out during the previous contract period will be continued with the objective of substantially reducing the radiation burden for diagnostic procedures in the young age, with particular reference to diagnosis and follow up of postural disorders (e.g. scoliosis), and of cardiac malfunctions. The work aims at the improvement of current methods for assessing radiation exposure in medical diagnostic procedures, and at the assessment of the radiation cost vs. diagnostic benefit of radiological and radioisotopic procedures, with particular reference to the work up of pulmonary, cardiac and renal patients.

1. Further development of methods for dose evaluation

   1.1. Development of the CAMIRD III for the internal dose evaluation after administration of radionuclides. Starting from CAMIRD III (the programm developed in the previous contract and now made available internationally trough BI-TIC Oak Ridge.. Tenn.) the goals for this part are :

   (a) to increase the CAMIRD capabilities with the inclusion in the basic software package of the specific absorbed fractions calculated for the heart : this will permit a more complete dosimetric assessment of the cardiological techniques,

   (b) to include the necessary modifications to take into account differences in body size;

   (c) to assess the evolution of the biokinetic radionuclide distribution using a Whole Body computerized gamma camera : this evaluation will require the use of suitable body and organ phantoms for calibration.

   1.2. Dosimetry in diagnostic radiology. This part specifically envisages :

   (a) continuation of the study aiming at the implementation of a computerized procedure for the dose evaluation to testes and ovaries, active bone marrow, thyroid, and uterus;

   (b) adaptation of the above program to account for differences in organ sizes;

   (c) completion of development, systematic use and assessment of a flexible procedure for the daily evaluation of the operators exposure in cardiovascular radiology.

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RADIATION PROTECTION PROGRAMME

Continuation contract no.: BIO-F-373-81-I

2. Radiation cost vs. diagnostic benefit in medical diagnostic procedures.
   2.1. The methods described in the previous section will be applied first of all to those Nuclear Medicine methodologies for which the patient radiation dose has not yet been evaluated and which are becoming of increasingly larger use, such as gating techniques and flow distribution using microspheres in cardiovascular medicine, lung ventilation imaging (inhalation of aerosols), positron imaging technique in both cardiac and lung studies (the cost of this new technique will be evaluated in light of the trade-off between the required information content at the tomographic section and the sensitivity of the detecting device). The radiation exposure assessment will be accompanied by a parallel investigation of the diagnostic relevance.
   2.2. Similarly, it is intended to complete the review of the most common X-rays procedures used for cardiac, pulmonary, renal and neurological patients work-up. It is hoped, to arrive at specific recommendations as to the relative merits of the various procedures entailing the use of ionizing radiations, taking into account the patients, the operators and the population.
3. Reduction of radiation dose to young subjects from medical diagnostic procedures. Investigations entailing the use of radiations in children or young adults, should be of great concern for possible effects on the individual and the population. Two areas, among others, show promise of good yield in terms of reduction of radiation burden to young subjects.
   (a) Cardiovascular angiography. This is often performed for suspected congenital or acquired cardiovascular disease : most of these procedures are based on the first transient of a bolus of contrast medium which is injected in order to delineate heart chambers and vessels. In comparison with the optimal time for visualization, a much longer irradiation time is usually applied : it seems possible that appropriate study of contrast transfer function, plus usage of iterative procedures for visualization of optimal cardiac cycle, might cut down a substantial part of the radiation exposure.
   (b) Spine diseases. Children and young adults; diagnosis and follow up of patients, mainly suffering from scoliosis usually implies repeated roentgenological studies of the entire spine, obviously entailing irradiation of gonads, plus marked all body radiation burden. Alternative approaches, such as stress analysis while walking or standing, with projection of stress vectors on the T.V. image of the subjects, in which critical points are identified by suitable markers, shows great promise of advantageous replacement of most of the X-rays follow-up of these patients. In cooperation with the Don Gnocchi Center in Milan, a comparative study of the effect on treatment will be performed in relation with the difference in radiation exposure.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-458-81-D

General subject of the contract:

Analysis of occupational exposure and exposure in medical diagnosis.

Description of research work:

1. Assessment of Occupational Exposure
   - Applied Dosimetry and Occupational Exposure Analysis.
   This investigation will be performed in close co-operation with the official dosimetry agency, which covers about 45 % of the personnel monitoring activity in Germany. The program is concentrated on designing of electronic data processing systems in order to set up data banks for demoscopic investigations of radiological data, details of the respective radiation installations, and results of dose measurements. With film dosimeters, additional information such as radiation energy and angle of radiation incidence is available and will be used to evaluate the monitoring results in terms of organs doses, effective dose and dose equivalent index, by means of suitable exposure models.
   In addition investigations will be performed on commercial protection level instruments (area and personal dosimeters) with the aim of modifying the energy and angular response functions in such a way that the dose quantities of interest in radiation protection will be directly assessed.
   - Calibration, Standardization and Intercomparisons
   The GSF has set up calibration laboratories, which represent regional reference installations for photon as well as neutron radiation fields. The photon calibration laboratory is a member of the IAEA/WHO network of secondary standard laboratories (SSDL). Both laboratories have already organized a series of international intercomparison studies. From the results it is strongly recommended that more intercomparison projects are performed in a continuing coordination effort.
2. Radiation Protection in Medical Diagnosis
   - Exposure Analysis in Medical Diagnosis
   Based on the hitherto existing experience with field studies and system analysis, test devices and procedures for determining the exposure conditions in common X-ray techniques will be developed which overcome shortcomings of former attempts. They have to be applicable in practice without disturbing the routine work too much and should record the physical parameters (radiation quality, irradiation geometry, film processing, image characteristics etc.) and provide further exposure parameters, e.g. entrance dose from

which the patient dose can be derived by means of suitable exposure models.

- Quality Control and Dose Reduction in Medical Diagnosis

Since the exposure analysis combines recording of physical parameters of the facility and the determination of patients dose, it offers starting points, for dose reduction. The user will be informed on the characteristic of his equipment, compared to standard conditions, as defined by the vast majority of participants. This allows to derive recommendations on how to improve his technics, standardize them, and check them routinely in order to reduce patient dose.

As in most diagnostic procedures image quality is correlated with patients dose, it seems worthwhile to combine a pilot study on the automatic recognition and classification of image quality.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-389-81-UK

General subject of the contract:

The use of cyclotron produced radioisotopes and tomographic recording procedures for studying regional tissue function in man.

Description of research work:

Radioisotopic tracers provide a means to study regional body functions. This concept has to date found widespread application in diagnostic medicine. As a result these procedures constitute a significant source of radiation load to the general population. This field is ever increasing and hence it is clearly important to assess the value of the emerging diagnostic and scientific information against the radiation absorbed doses.

At the Medical Research Council's Cyclotron Unit there exists a programme of research concerned with the application of radioisotopic techniques to problems in clinical Medicine and research. This is believed to represent the growing edge of this field in that the most advanced facilities exist in this institute. These consist of a source of short lived isotopes from the Hammersmith Cyclotron and tomographic Scanner which currently represents the most accurate instrument for measuring the uptake of tracers within the tissue of man. These facilities are combined with an active research team of Medical Doctors, Medical Physicists, Radiochemists, Engineers and Computer experts.

During the period of the contract the laboratory is committed to projects concerned with the study in health and disease of regional cerebral, cardiac, pulmonary and abdominal tissue. It is proposed during this programme to use the Euratom contract to extract a cost benefit analysis from the emerging data. This will involve an assessment of what new diagnostic and scientific information can be obtained and how accurate, relative to the associated radiation load to the patient. Although currently specialised work, an analysis of the relevance of this field is believed important to the Community since the number of facilities similar to those at Hammersmith are increasing within Europe.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-368-81-D

General subject of the contract:

Effective dose equivalent due to X-ray diagnostic procedures and dose equivalent resulting from natural radiation exposure including the enhanced technological exposure.

Description of research work:

For radiation protection purposes, the International Commission on Radiation Protection (ICRP) has introduced, in its recent publications, the concept of the effective dose equivalent, together with the recommendation to embody this concept into the supranational and national radiation protection standards. Accordingly, the review of the standards is going to be prepared by EURATOM and by the authorities in the Federal Republic of Germany. In view of this programme, the Institut für Radiologie proposes two subjects relating to radiation protection.

The first part of the programme considers the population exposure and risk assessment in X-ray diagnostic procedures.

(a) In this part it is intended to measure the dose distribution in the body with various X-ray diagnostic procedures. The aim is to derive the effective dose equivalent due to the exposure of tissues at risk, according to the new ICRP concept. Dose distributions in X-ray diagnostics are measurable only in phantoms. Here, an ALDERSON-Random-Man phantom will be applied. The dose measurements will be done by thermoluminescence dosemeters, pecularly developed in this laboratory, throughout a three-dimensional lattice. From the dose distributions, the absorbed energy (integral dose) can be determined for the domain of the tissue at risk. This may conduct to a practicable and sufficient estimate of the effective dose equivalent.

(b) The statistical data on X-ray diagnostic procedures, as known from the literature, have to be completed or will be exemplified; respectively, over the urban agglomeration Nürnberg - Fürth - Erlangen. The diagnostic procedure parameters are subject to large variations from one radiologist to another and from one equipment to another. Therefore, the influences of these variations on the effective dose equivalent values will be examined. From the variation range, conclusions leading to a reduction of the exposure may be drawn.

(c) From the effective dose equivalent values and their variation ranges and from statistical data on the various X-ray diagnostic procedures, the effective per-caput dose equivalent and a malignoma significant dose may be derived, according to the concept of the ICRP Publication 26. It is intended to determine, with the aid of estimated values for the individual procedures, such types of examinations which give rise to the greatest contributions to the effective dose equivalent, in order to emphasize distinct diagnostic examinations and

distinct age groups, in analogy to the procedure applied to the genetically significant dose.
The aim of the second part of the programme is the determination of the mean effective dose equivalent resulting from natural radiation exposure and of its frequency distribution for the population of the urban agglomeration Nürnberg - Fürth - Erlangen. These values may serve as a reference for the risk evaluation of X-ray diagnostic procedures (see part 1). The measurements will be done by use of CaF_2 -TL-dosemeters according to two different methods :
(a) The exposure rate profile throughout a representative measuring location grating will be determined in free land and in urban environment and on a representative random sample of dwellings and buildings. Statistical random sample data or estimate of the occupancy density and occupancy time of the members of the public at the various location types which can be formed according to the exposure rate values will be collected. The mean effective dose equivalent and its probable variation range will be calculated.
(b) Personal dose measurements throughout a statistically significant random sample of the population will be carried out. The population structural data and, from this, the required magnitude of a random sample and of its structure will be evaluated. CaF_2-dosemeters, together with an appropriate questionnaire, will be distributed to volunteers selected according to the random sample structure. A statistical evaluation of the recorded dose values of the questionnaires and conversion of the distribution of the recorded dose values to the global and the structured frequency distribution of the effective dose equivalent will be done.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-387-80-UK

General subject of the contract:

Measurement of doses to patients from the medical uses of radiation.

Description of research work:

The importance of monitoring radiation doses associated with health care stems from the fact that for most developed nations, medical irradiation (especially diagnostic radiology) is the largest man-made contributor to the dose to the population. The present project will complement a recently completed study of the genetic dose arising from diagnostic radiology in Great Britain.

The complex nature of diagnostic X-ray examinations, involving partial body exposure, together with the possiblity of field size, position and direction changing several times during the procedure, makes the routine assessment of somatic doses difficult to accomplish on a large scale. Whilst the method of direct dose measurement with TLD sachets attached to the patients skin has been used successfully in studying gonadal doses, this technique does not easily lend itself to determining mean doses for other more spatially distributed organs of the body such as the bone marrow. As an alternative to the mean organ dose, the concept of the total energy imparted to the patient during an X-ray examination may be used as an index of relative somatic hazard. This approach has the advantage of being equally applicable to all types of examination, and may easily be implemented in practice on a routine basis using a "Diamentor" large area parallel-plate ionization chamber monitoring the total output of the X-ray set, provided a suitable calibration can be established.

The basic function of the "Diamentor" is as an indicator of the area exposure (R x cm2) of an X-ray beam. Its calibration in terms of energy actually imparted to the patient will involve both experimental TLD studies of the integral dose deposited in an Alderson Rando anthropomorphic phantom during simulated examinations, together with Monte Carlo calculations relating the fractional energy of the beam absorbed in an homogeneous mathematical phantom under various irradiation conditions. For given operating conditions of an X-ray generator, direct estimates of energy imparted should then be possible from a "Diamentor" reading, allowing data for the various major radiological procedures to be accumulated conveniently on a national scale. Such a compilation may then be used for the purpose of comparison and the evaluation of somatic risk.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-424-80-UK

General subject of the contract:

The development of methodologies for evaluating and controlling the risks associated with planned and accidental releases of radioactivity.

Description of research work:

The release of radioactive materials from nuclear installations in either normal operation or accidents will result in the exposure of the population and contamination of the environment to varying degrees. The evaluation of this exposure and contamination in particular circumstances fulfills a number of important roles including :

- the estimation of the radiological impact and risk presented by the installation
- pre-planning emergency arrangements in the event of accidents
- an input into the optimisation of waste management practices
- an input into decisions on the siting of nuclear installations

The objectives of the programme are to develop methodologies capable of broad application and to demonstrate their application in each of the above areas. The programme can be divided into two separate topics and the content of each is summarised.

1. Methodology for accidental releases and its illustrative application.

   The radiological impact of accidental releases of radioactive materials can be categorised as follows :

   - early biological effects in the exposed population
   - late biological effects in the exposed population and its descendants
   - the contamination and potential restrictions on the use of agriculture produce.
   - the contamination and potential restrictions on the use of property and industrial premises.

   The methodology to be developed will enable the respective contributions to the radiological impact to be assessed within a common framework. The relative importance of the respective contributions and how they might interact with design provisions for, and the siting of, nuclear installations will be investigated. The sensitivity of the results to a range of assumptions as to the relative weighting to be assigned to accidents of different magnitude will be analysed.

   The methology will be applied to a number of representative accidental releases to illustrate its use in the formulation of emergency arrangements.

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2. Methodology for the evaluation and control of exposure from effluents during normal operation.
Existing methodologies for the estimation of the radiological impact of effluent discharged during the normal operation of nuclear installations contain a number of conservative assumptions. To enable more realistic estimates these methodologies will be refined and extended to enable the distribution of the collective dose in the exposed population to be determined in individual dose ranges.
The role of collective dose as a measure of health detriment in the optimisation of waste management practices for and in the control of airborne and liquid effluents will be examined for a range of practical situations. Particular attention will be given to three aspects :
- the relationship between collective dose and maximum individual dose and their relative significance
- the influence of the distribution of the collective dose in the exposed population
- the influence of the temporal distribution of the collective dose

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Head(s) of research teams(s):

Contract no.: BIO-F-480-81-I

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General subject of the contract:

Assessment of the indoor dose in Italy.

Description of research work:

The indoor dose may be considered one of the main contributions to the natural dose received by the members of the general population.
The assessment of the indoor dose in Italy should be considered of great significance owing to the particular geomorphological characteristics of the country. It has been demonstrated that large areas (Lazio, Campania) exist in Italy where high external natural doses are received by the local population.
The extensive use of building materials (vulcanic tuffs) originated in those areas seems to indicate that the indoor dose may also assume a particular relevance.
The indoor dose will be evaluated by taking into consideration the two following items :

- the external irradiation inside the houses due to the photons emitted by the natural radionuclides which are contained in the building materials.
- the internal irradiation due to the inhalation of radon and daughters released by the radium content in the building materials. The thoron and daughters will be also considered in those cases where the thorium content in the building materials is of particular significance.

This task will be achieved by measuring :
1) The external dose inside the house by means of TLD dosimeters particularly designed for such purpose.
2) The radon and thoron daughter concentration in indoor air by means of well known standard methods (e.g. Markov and spectrometric) and by a recently developed method based on a track etching detector (CR 39). All these methods are applied to the measurement of radon and thoron daughters deposited on filter spot samples. The choice of the analytical method will be decided according to the experimental requirements.
A CR 39 passive detector is also under development to be located for long periods inside the houses as an integrated detector of the indoor total alpha activity air concentration.
3) The radon concentration in indoor air by means of a ZnS scintillation chamber and of the two filter method.
4) The radon exhalation rate from building materials and walls of dwellings by means of the radon build-up technique in a closed vessel.

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5) The concentration of natural radionuclides (226-Ra, 232-Th and 40-K) in building materials by means of gamma-ray spectrometry based on a Ge (Li) detector and an on-line computer multichannel analyzer.
6) The size distribution of the radon daughter aerosol by means of an inertial spectrometer coupled to track etching detectors (CR 39).
All these measurements are intended to correlate the internal and external dose absorbed by subjects living inside well selected dwellings to the radiological characteristics of the materials used to build the dwellings. A careful consideration will be given to all the parameters, as occupancy factor, ventilation rate, seasonal factors etc., which are of particular importance for the assessment of indoor exposure.
A large number (about 1000) of dwellings located in the various regions of Italy which may be considered representative of the areas showing low, intermediate and high natural dose levels will be considered for the indoor dose measurements over a prolonged period of time (over three months) by means of TLD and CR 39 track etching detectors. A more restricted number of dwellings particularly representative of all examined dwellings will be subjected to a carefull follow-up for a complete characterization of the exposure.
All the building materials related to the considered dwellings will be measured.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-320-81-F

General subject of the contract:

Comparative study of the impact of conventional and nuclear industries on the environment and the public from the twofold standpoint of radioactive and chemical releases.

Description of research work:

The study to be carried out will make it possible to assess and localize in an objective manner the extent of the hazards and associated detrimental effect which are inherent in nuclear or non-nuclear industrial activities, among all the hazards to which the population of a given region is exposed. In addition to providing information in the field of risk comparison, both in respect of the methods used and with regard to risk evaluation, the study will also constitute a model capable of being transposed to other regions in Member States of the European Community. The industrial activities to be covered will be the electricity generating industries (coal and oil-fired thermal power stations, nuclear power stations), the activities associated with them (extraction and processing of raw materials, processing and storage of waste, etc.) and certain industrial activities which are sources of pollution (refineries, chemical industries, iron and steel industry, etc.) The principle of this study will consist in associating theoretical evaluations based on estimation models with a programme of in-situ observations. The region chosen for this study is situated in the south-east of France (Greater Rhône Delta) and almost all the industrial activities referred to above can be found there. In the initial stage, the exact limits of the region to be studied, the industrial activities to be covered, their location and the principal characteristics with regard to supplies for them, their functioning and their production will be defined. A survey will also be made of the environmental features of the region under consideration, mainly in respect of meteorology, hydrology, pedology, the agricultural economy and the population distribution. Existing information on the pollution levels, whether radioactive or not, measured in that region will be compiled.
The second stage will consist of the preparation and implementation of the corresponding sampling and measurement programmes. These will concern:

- the source factors (measurements in respect of the raw materials used, the effluents discharged into the environment and the residues);
- the environment, both the physical environment (atmospheric gases and aerosols, deposits, surface waters, soils, sediments, etc.) and the biological environment (plant and animal production, food products, etc.).

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The pollutants to be measured will be chosen in accordance with the activities under consideration, the extent to which they occur in the discharges or the residues and the potential nuisance that they represent.

The following pollutants, in particular, will be taken into consideration :

- artificial radionuclides (fission or activation products);
- radioactive elements belonging to the natural chains (uranium, radium, etc.);
- dusts, sulphur and nitrogen oxides;
- certain heavy metals;
- organic compounds, particularly those which are suspected of or are recognized as being mutagenic (polycyclic hydrocarbons, ethylene, organic molecules, etc.).

The programmes will deal in succession with :

- discharges into the atmosphere from industrial electricity-generating facilities;
- discharges into the atmosphere from the industrial facilities associated with the preceding ones;
- discharges into surface waters from all of the facilities referred to above;
- discharges into the atmosphere from other industrial facilities not generating electricity;
- discharges into surface waters from the last-mentioned facilities.

In all cases, the discharges are associated with the normal operation of the various facilities. Simultaneously, the dispersion models will be applied to the various source factors, the data from the literature supplementing those obtained by means of the measuring programmes, and the impact zones in the region under consideration will be determined.

The available data relating to occupational hazards in the industrial sectors concerned will be compiled, whether or not these hazards are radiological. Finally, a study programme intended to define the most appropriate hazard indicators and how to express them in terms that make it possible to perform a comparison between hazards of different types will be developed.

A preliminary analysis of the results obtained in this study and of those that will have been obtained in the associated programmes will be performed for the purpose of revealing the remaining shortcomings and gaps.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-444-81-F

General subject of the contract:

Analysis of occuptional exposures and diagnostic medical irradiations.

Description of research work:

The ultimate objectives of the research programme consist in establishing the data bases necessary for an improved assessment of the radiological risk related to artificial sources. The two activites selected are those which are at present the main sources of external irradiation. In the case of workers in the nuclear power cycle, the power stations are concerned while in the case of the population it is medical irradiation for diagnostic purposes. It appears, moreover, that the collective doses corresponding to the industrial and medical practices involved could be reduced by adequate protective measures which will be described later on.

1. Evaluation of occupational irradiations in PWR power stations. Recent estimates of the occupational exposures in the nuclear power cycle suggested that the power stations contributed extensively to the total of the corresponding collective doses. This was caused both by the number of workers concerned and by the average doses per worker. In view of the fact that little experience has yet been acquired in Europe in operating PWR power stations, only rough theoretical estimates are available at present concerning the contribution of the various operating, servicing and maintenance activities to the collective dose. Most of the data are still provided by the USA, but these results are difficult to interpret and even more difficult to transpose to European circumstances since the doses reported are not backed up by a detailed description of the operating incidents and of the particular difficulties encountered during maintenance work. These reasons prompted the french operator to set up various specific systems for collecting data. These data concern routine operating situations, mainly the conditions under which the personnel performs its work during cold shutdowns. It is proposed to draw up a balance sheet of the various sources of information which are available or are being set up in the French power stations, and to compare the results with equivalent data. It is intended at a later stage to perform appropriate statistical analyses of these data in order to interpret them in terms of defining the activities or the places for which special radiological protection measures should be taken. The data fall mainly into the following distinct categories :

- dose rates at different locations related to the parameters describing the status of the power station;
- measurements of atmospheric contamination;

- measurements of the primary water activity;
- collective doses per operation;

It should be pointed out that, during 1981, the first data collected by an automatic read-out system for dosimetric measurements, which is being developed at present, will become available. This system has been designed to enable daily individual measurements of the dose received by both the statutory workers and the emergency operations staff and to relate the dose to the tasks performed. At the end of 1980, the system will become operational for certain power stations.

2. Diagnostic medical irradiation.

Data on the irradiation of the public caused by diagnostic medical irradiations are rarely compiled in a systematic manner. The information provided by the reimbursement statistics of the social security systems is limited to consolidated financial data unsuitable as bases for reconstituting the relevant medical acts. However, these data clearly indicate a continuous and rapid increase of these acts presenting problems with regard to the collective exposure, the quality of the diagnostic methods and the associated cost. Moreover the corresponding irradiations of the public are difficult to assess because the calibration of the equipment is not methodically checked everywhere. For these reasons, it is planned to carry out a sample survey among the various medical services and private offices making use of radiological equipment. The survey will initially be limited to diagnostics in urban practices and medical institutions, and will not include systematic examinations at schools, at work, at the army, etc... The intention is to question a representative sample of physicians, performing radiological acts, on their medical activities over a period of several days (prospective survey). A distinction will be made between radiological acts performed on an out-patient basis (radiologists and, other specialists making use of radiology) and in medical care institutions.

The intention is to prepare a questionnaire containing a detailed nomenclature of the radiological acts performed, the reasons for the examination, the origin of the prescription, the patients' characteristics, etc. Furthermore, it seems necessary to check the calibration of the equipment by giving a film test to the practitioner. After adaptation to the national level, the data acquired may make it possible to obtain an irradiation balance-sheet related to the different types of diagnostic examinations and to the current different medical practices and would serve as a basis for a feasibility study of reducing doses and evaluating diagnostic methods.

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Head(s) of research teams(s):

Contract no.: BIO-F-445-81-F

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General subject of the contract:

Radiodiagnostic irradiation of men and reproduction.

Description of research work:

The present programme is a feasibility study previous to a research programme which aim is to obtain relevant informations relative to the following questions :

1) What is the individual statistical distribution of the number and type of radiodiagnostic examinations performed on men who can potentially conceive children ?

2) Which proportion of men who can potentially conceive children and were exposed to radiodiagnostic examination do actually conceive within 3 months following this examination ?

3) When radiodiagnostic examination of a man is followed within 3 months by conception, is there any increase of the incidence of reproductive failures ?

The originality of the approach proposed results both from the consideration of all radiodiagnostic examinations performed on all the men of a non selected population and from the definition of the concept of a critical period of three months following such examinations. This last concept underlines the difference which should be considered when comparing potential reproductive hazards resulting from radiodiagnostic examination of women, whose gametes are available since their birth and those which can result from the exposure of men, who produce new spermatozoids continuously during the spermatogenesis, which lasts 2 to 3 months.

1. Radiogiagnostic examinations of men which can potentially conceive children.

   The "Caisse Primaire Centrale d'Assurance Maladie de la Région Parisienne" centralizes the requests of reimbursement of all the medical acts concerning 5 million people living in the Paris region. This instituion is therefore led to manage on a computer system several millions of requests of reimbursement each year.

   Several hundred thousands of demands of reimbursement concern radiodiagnostic examinations of men aged between 20 and 45 years. Each one of these demands contains informations allowing to identify the patient, to date and describe the examination, performed (speciality of the physician performing the examination, number and "coefficient of reimbursement" of the examinations, examination performed during hospitalization or not). The "Caisse Primaire Centrale d'Assurance Maladie de la Région Parisienne" allowed to gather and analyze these data. It will therefore be possible to

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analyze the individual statistical distribution of the number and type of radiodiagnostic examinations performed on men of the specified age, particularly as a funtion of their age. Also the identification of the men examined will allow to make the link between those data and informations concerning gestations these men fathered.

2. Investigations concerning different possibilities to link the radiodiagnostic data with gestation data.

Three different possibilities to link the preceeding radiodiagnostic data with data concerning gestations will be explored :

(a) Use of the data of the "Caisse Primaire Centrale d'Assurance Maladie de la Région Parisienne" which reimburses the "First Prenatal Medical Examination" performed prior to 4 months of gestational age and/or reimburses the expenses corresponding to the bringing forth of the child.

(b) Use of the data of the "Caisse d'Allocations Familiales" which is responsible for the payment of the familial support given to the families. This institution also gathers informations concerning the "First Prenatal Medical Examination" and the issue of the corresponding gestations.

(c) Use of the data of the Ministry of Health which centralizes the first certificates of health to be filled in for all children within 8 days after birth.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-F-422-80-D

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General subject of the contract:

Quantification of radiation risks.

Description of research work:

It is the aim of this work to quantify and evaluate the radiation risks of low doses and dose rates for the general population and for particular groups. In particular this project is concerned with those risks which are due to those radiation effects which are due to radiation exposures slightly elevated above normal by technological or civilisatoric measures. The results of the analytic phase of this work will be applied to special practices or sources as e. g. to radioactive emissions from nuclear power stations, to exposure of the population by medical X-ray diagnosis, and to exposure from radon daughter products in houses.

To this purpose, first the dose distribution in a population and its dependency on age, sex, time, etc. will be estimated. As regards exposure from radionuclides, the time dependent radiation exposure in humans after ingestion or inhalation of nuclides will be calculated by stochastic computer simulation programmes. Hereby also non-standard conditions will be taken into account if sufficient data become available. Special emphasis will also be put on the biological variation of these various exposure values.

Secondly, available epidemiological and other relevant sets of data will be analysed with respect to the time dependence of the incidence rate of health effects due to these low level exposures. To this purpose a number of well established as well as new developed statistical techniques will be applied to these data to investigate their consistency with certain assumptions presently used in radiation risk estimates (e.g. relative or absolute risk models, multivariate dependency of latency periods, etc.).

Finally, an attempt will be made to improve present methods used in radiation protection to measure consequences of exposure, e.g. in specification of "risk", in benefit/detriment estimates, and in optimization studies (see e.g. ICRP-Report 27). To this purpose, mainly the possible advantages in risk intercomparisons of the quantity "loss of healthy life span" and of derived quantities will be assessed. These quantities will be calculated for selected cases using the multivariate risk functions derived in the second phase of this work, and consequences analysed of their use e.g. in the assessment of the detriment to individuals and populations.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-319-81-F

General subject of the contract:

Study of the various components of natural irradiation associated with the dwelling place.

Description of research work:

The evaluation of the natural radioctivity and of the increase of natural exposure associated, in particular, with the dwelling place must be performed in the most realistic manner by attempting to make the best possible determination of the various components.
The purpose of the proposed study is to evaluate the contribution of the dwelling place to the irradiation of the public from two points of view :

- external irradiation due to photons emitted by natural radionuclides contained in building materials;
- internal irradiation due to the inhalation of radon and its daughters produced by the radium contained in the building materials.

To attain this objective, it is necessary :

- to measure the irradiation levels within dwellings, whether traditional or new;
- to measure the radon and its daugthers in the same dwellings;
- to analyse the various building materials with regard to their type and the origin of the raw materials used for the purpose of determining the activity of the different natural series;
- to measure the irradiation levels in dwellings constructed with those types of material which have previously undergone laboratory analysis.

The procedure adopted to fulfil these different tasks consists in :

- measuring the external irradiation by means of thermoluminescent dosimeters; dubious measurement results are checked by means of dose-rate measurements with a calibrated organic scintillator;
- measuring radon by means of a S-Zn scintillator used on on-the-spot samples;
- measuring radon and its daughters trapped on filters by reading the traces on cellulose nitrate;
- analysing the building materials by means of fluorimetry and spectrometry.

All these measurements should make it possible to correlate the external irradiation and the measurements of radon concentration with the types of building materials.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-F-335-81-UK

General subject of the contract:

Environmental factors influencing the doses from inhaled radon daughters.

Description of research work:

The evidence linking an excess incidence of lung cancer with high exposure to radon daughters in uranium and other mine air is incontrovertible. From the studies of such excess lung cancers in uranium miners a risk estimate for lung cancer induction per unit radon daughter exposure can be derived. Radon-222 and its daughters exist in the general environment out of doors and at higher concentrations within buildings. Thus all members of the population receive a radon daughter exposure, albeit much lower than that experienced by miners. It is likely that a small fraction of lung cancers in the general population result from this radon daughter exposure. Estimates of population radon daughter exposure in the UK and other countries have been made. It is extremely unlikely that the risk estimate derived from miner studies can be applied to the general population as the pattern of radon daughter deposition in the lungs of the two groups will not be the same.

A critical parameter in determining the deposition pattern of radon daughters in lung is the aerosol size distribution in the atmosphere. Aerosol size distributions in mines have been studied but there is very limited data on the size distribution in the normal atmosphere within homes and work places other than mines. No such data exists for the UK.

It is proposed to measure the size distribution of aerosols in dwellings, offices, other work places and in outside air. Measurements will be made in urban areas and the effects of common atmospheric pollutants such as cigarette smoke will be studied. The overall aerosol size distribution may not coincide with the size distribution of aerosols to which radon daughter attach. It is proposed to determine the sizes of aerosol to which radon daughters preferentially attach and the fraction of radon daughters existing as free ions or atoms in the environments discussed above.

The main instrumentation to be used in this study will be a 10 stage diffusion battery which will permit classification of aerosol particles in the range 0.002 - 0.2 μm. The device will be used in conjunction with an automatic switching unit and a condensation nucleus counter. This enables the condensation nucleus concentration upstream and downstream from each stage of the diffusion battery to be measured in sequence and from the results the size distribution of the aerosol in

the atmosphere can be determined.
When typical size distribution of aerosols in the atmosphere have been determined a much higher concentration will be generated in an environmental chamber in which the radon concentration in air will be much enhanced. Although a higher condensation nucleus concentration than is usual in the general environment will be generated, the size distribution will be matched to that of the environment of interest. By repeating the experiments outlined above but with the radon daughter concentration much elevated, sufficient radon daughter activity will collect on the fine wire mesh screens which form the diffusion battery stages. This activity will be measured when the screens are removed. In this way the size distribution of aerosols to which radon daughters attach will be determined.
The free, or unattached, fraction of radon daughters will be measured using a dual sampling and detection system. One system will be preceded by a parallel plate diffusion battery to remove the free fraction. Alpha spectrometry will be used to measure the radon daughter concentrations recorded by each detector.
The determination of typical free fraction and aerosol size distributions for radon daughters in the normal environment will be used by another group in NRPB to calculate the relative deposition per unit radon daughter exposure for members of the public compared with uranium miners. The objective is to provide a firm basis for modifying the risk estimate derived from miners to produce a more realistic risk estimate to be applied to the general population.

Commission of the European Communities

RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-337-80-UK

General subject of the contract:

Quantification of risks associated with ionizing radiation using data from human studies.

Description of research work:

The analysis of hazard associated with exposure to ionising radiation has various aspects, none of which are free from dispute. In this proposal, it is intended to consider three main projects :

(1) Data is being collected in the United States and in Britain on the occupational exposures of radiation workers and these will be correlated with mortality by large, long-term epidemiological studies. These data collections are the most direct way of obtaining evidence on the effects of low levels (occupationally permitted) of radiation. Because the effects are small compared with the number of the same effects which are expected in any unexposed population, sophisticated statistical procedures must be employed in attempts to resolve any possible excess among an exposed population. Therefore, it is desirable that progress with different studies is monitored so as to maximise not only the information from each separately but to look for supporting conclusions between studies which may increase the significance of any findings or identify any spurious results. In advance of analyses of these data, it would be useful to investigate what may be expected from the studies and what might be able to be done with the results when they become available.

(2) To interpret the data on which risk estimates are based, models are necessary. There are difficulties with even the simplest expressions of these models. For example, the BEIR model for relative risk is $R = Ro\ (1+ gD)$ where R is the risk when exposed to a radiation dose D if the risk without the exposure was Ro; g is a constant. In this expression Ro is not the risk at the time the dose is incurred but a future risk at a time when the dose may be effective. Thus, there are ambiguities of age at exposure or age at expression, to resolve. Alternatively, there may be written an incremental form of the relative risk model $dR = gRdD$ where dR is the increase in risk for an increment of dose dD. In this expression the g is not a simple quantity. The attractiveness of an absolute risk model is marred by the lack of formal statistical procedures for its use.

The survey of the models that have been used and a formal treatment of risk theory incorporating long latency is essential. It will entail drawing together radiobiological, epidemiological and statistical expertise in an exploration and clarification of the application of the available models.

(3) Finally, it is of little value to examine a risk in isolation from other risk. Comparative risk analysis is made difficult by the different characters of risks that may result from alternative methods of achieving the same benefit. Such a comparison would be feasible if an index were available to compare fatal accidents, late effects, morbidity and in general any detriment to the quality of life. ICRP 27 discuss some of the difficulties and outlines a possible procedure. This averages effects and since distribution of risk can be crucial to the outcome, it is desirable to look in more detail along the lines of the references below based on life shortening.

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Head(s) of research teams(s):

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Contract no.: BIO-F-369-81-D

General subject of the contract:

Research Project "Thorotrast" Investigations to evaluate the long-term effects caused by artificial radiation in man (thorotrast patients) - Follow-up study.

Description of research work:

The German Thorotrast Study, a supraregional research program, was started in 1968. From the records of different German hospitals the names and the addresses of 5.159 Thorotrast patients were ascertained. In order to compare the patients of the Thorotrast group with the general population a pseudo-randomized selected control group (N=5.158) from the same hospital was investigated. When the research program was started, many of the primarily identified patients had already died. An attempt was made to find out the time and the cause of death of all deceased patients. Those patients who had died within 3 years after the injection of Thorotrast, respectively those patients of the control group who died within 3 years after the hospitalisation were excluded from evaluation. A large number of patients primarily identified were lost for follow-up investigations for different reasons.

Furthermore 901 Thorotrast patients and 676 patients of the control group were examined clinically and biophysically. A complete clinical status with laboratory tests and x-ray examinations was carried out in each case. The biophysical examination includes whole body counting and measuring Thoron concentration in the breath. Additionally the upper abdomen was examined by ultrasonography, scintigraphy and computed tomography. Therefore excellent information of anamnesis, clinical status and the amount and distribution of Thorotrast in the body are available. The examined patients of Thorotrast and control group are contacted every year and invited for reexamination every second year (Follow-up study). Close contact is kept to the family physician, who obtains all examination results. By a special examination program during 1979, 20 Thorotrast induced liver tumors could be detected and partially could be removed surgically.

This procedure proved to be the best way to give medical help to the Thorotrast patients and to obtain best epidemiological results. The final fate of deceased patients is investigated. The annual rate of liver tumors and leucemias is still increasing. By the end of 1979, 1042 patients of the Thorotrast and the control group are alive. It seems to be urgently necessary to continue the follow-up of these patients in the above described successful way. These patients are exposed to Alpha-radiation over a period of time of more than 35 years. Their fate will be of great influence to the final results and consequently to the conclusion of the study.

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RADIATION PROTECTION PROGRAMME

Continuation contract no.: BIO-F-369-81-D

Summarizing the research project Thorotrast (Follow-up study) includes :

1. Biophysical investigations to calculate the total effective radiation dose caused by the Thorium dioxide deposits.
2. Clinical examinations to determine the state of health.
3. Use of immunological methods, ultrasonography, scintigraphy and computed tomography for the discovery of Thorotrast-induced neoplastic lesions.
4. Classification of the cause of death for patients who had died since the last examination.
5. Identical studies of the control group.
6. Statistical analysis of the results obtained.

Apart from this epidemiological study, the following investigations are part of the program :

1. Animal experiments to investigate the distribution of the Thorium dioxide particles within the body and to calculate the resulting radiation dose to the tissue.
2. Animal experiments to estimate the non-radiation effects of deposited Thorium dioxide.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

Contract no.: BIO-F-423-81-F

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General subject of the contract:

Methods for evaluating the consequences of irradiation of the public

Description of research work:

The purpose of the research programme is the adjustment of methods for a correct and consistent evaluation of the irradiation of the public and the environment within the European Community.
The aim of the first part of the study is to continue the objective adjustment of methods for evaluating individual and collective doses resulting from various types of exposure to ionizing radiation. These methods are applicable on a European scale and assess the consequences of the irradiation of man and his environment, bearing in mind the diversity and the variability of the characteristics of the receiving environments and of the sources.
The problems peculiar to the discharge of radioactive effluents into the atmosphere were particularly studied in the preceding contract, but the methods developed and the information acquired or sought were intended for application to various sources. The methods will be supplemented and tested by means of application exercises in a European context. Taken into account the work performed and the present state of knowledge, the orientations will be as follows :
1. Atmospheric discharges - A detailed analysis will be made of the results of application exercises for providing the necessary additional data in order to evaluate the consequences of accidents according to different scenarios. The studies will be based on accident hypotheses and existing situations in order to serve as guides to the estimated evaluations used in the preparation of reports required in compliance with the procedure laid down in Article 37. With this in mind, a particular study will be made, of the dose distibution within the European population and its evolution with time.
2. Liquid discharges
a) A critical examination will be made of the results of the application of the models in extended aquatic environments. This examination may eventually lead to a study of an additional programme of appropriate measurements to be carried out in the framework of a co-operation with competent European laboratories.
b) The study of the changes of the physico-chemical form of plutonium and the transuranic elements in the environment which was started under the preceding contract will be continued. In collaboration with

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specialists of marine and terrestrial radioecology, and as a part of the Eurosoil project, this study will be extended to the terrestrial environment.

3. Methods for evaluating the balance-sheets of exposure of the European population - Data regarding the comparison of different sources of irradiation will be methodically collected and the information will be presented cartographically in a way similar to the one for evaluating the consequences of discharges from nuclear facilities. The methodologies already developed will be used for processing those data likely to enable the establishment of balance-sheets of the irradiation of the European population.

The second part of the work is the development of methods for evaluating the radiological detriment and its cost. The evaluation of stochastic and non-stochastic effects of irradiation will be carried out in accordance with the three following complementary principles :

1. Implementation and use of epidemiological studies carried out on populations exposed to various sources of irradiation. Three approaches will be developed :

(a) Analytical epidemiological studies of populations subjected in the past to therapeutic irradiation. Some of these studies will aim to specify the nature of the interactions between the ionizing radiation and various risk factors (additional, synergy). Part of this work will be devoted to the study of genetic effects.

(b) Epidemiological studies of occupational exposure to ionizing radiation. The balance-sheet will be established and an attempt will be made to coordinate and harmonize the health records of this category of workers.

(c) Study of the natural irradiation. The establishment of a balance-sheet of the exposure of the public to natural irradiation will be completed by the implementation of a programme of measurements for the evaluation of domestic irradiation.

2. Use of data from animal experiments in view of the establishment of a model for radiation-induced effects.

3. Theoretical studies in order to establish more realistic models for the dose-effect relationship models which can be proposed to the authorities responsible for radiological protection.

4. In addition, the studies concerning the cost of radiological detriment will be continued by developing methods for evaluating the economic and social consequences of irradiation. Studies will be devoted to the determination of the implicit costs of prevented health effects in various industrial activities. This work will necessitate the development of methods to place the levels of residual risk reached in various industrial sectors in the context of their socio-economic importance.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-F-423-81-F

sub-contract : SC-002-F

General subject of the contract:

Contribution to the study of medical irradiation : systematic diagnostic radiology in France.

Description of research work:

The main source of irradiation of the population, in connection with human activities, is the use of ionizing radiations for medical diagnosis. The constant increase in the actual amount of radiology being carried out over the last fifteen years in all countries of the European Community poses the problem of assessing, monitoring and reducing the radiological dose absorbed by the exposed population. The purpose of the proposed programme - an extension of a study currently being carried out - is to collect and synthesize data available in France in relation to systematic radiological examinations for detection, monitoring and recruitment purposes.

Examinations of this type are carried out in the context of numerous public and private institutions without the existence of an overall standard accounting system. It is not even possible to obtain general accounting data since such examinations are not registered by the Health Insurance reimbursement services.

It is proposed that data be collected from the principal institutions concerned :

- school and university medical services,
- military medical services (selection, recruitment, monitoring),
- industrial medical services (recruitment and monitoring),
- services responsible for carrying out medical examinations under the French regulations governing Health Insurance.

These data would relate particularly to : a) the annual activities of the services (number and type of examinations) ; b) the groups of the population examined and their demographic characteristics (age, sex, usual place of residence, etc) ; c) the radiological equipment used, in particular the relative importance of the radioscopy ; and d) radiological exposures (part of the body examined, number of radiographs).

The data should be supplied together with all details useful for processing which will enable them to be represented in an existing grid model of the European Community. It is planned not only to collect the most recent data available, but also to look for the most appropriate guidelines for a follow-up to the information in question.

Particular attention will be given to industrial medical services in view of the range of different systems used for monitoring workers. The analysis will be concentrated on specific regions in order to test the

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RADIATION PROTECTION PROGRAMME

Continuation contract no.: BIO-F-423-81-F ; sub-contract : SC-002-F

validity of the proposed data collection system. Such an assessment will in the first place enable to compare the level of irradiation in various groups of people, and secondly, will contribute to consider possibilities of reducing the dose in systematic radiological examinations. In the long term it will contribute to the establishment of medical irradiation mapping at the European level and will permit to analyse and assess the value of this form of prevention.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-F-423-81-F

sub-contract : SC-003-F

General subject of the contract:

Research to define the implicit values of an occupational health hazard : methodological approach applied to uranium extraction.

Description of research work:

The extraction of uranium ore in non-sedimentary mines is the stage in the nuclear fuel cycle that involves the highest average risk for the individual worker. There are not only the traditional accidental risks inherent in underground mining, but also those arising from the inhalation of dust, daughter products of radon and exhaust fumes from machinery, and lastly from external radiation.

In the context of the future implementation of the ICRP's recent recommendations concerning new methods of calculating the limits of intake of radionuclides, it is appropriate to envisage the various means of protection which would make it possible to reduce to a greater extent the doses received by uranium miners.

A former study carried out in 1980 indicated the importance of primary and secondary ventilation with regard to reducing alpha-contamination. A number of options, most of which are currently being studied and developed would make it possible to concentrate on ventilation systems and to improve their performance : thus, for example, turbulators which prevent the air inside the mines from becoming stagnant, or filters purifying the primary air penetrating into the secondary ventilation system.

It is intended to study the implicit costs of the health hazard in connection with the various possible options for reducing the doses received by miners. A first stage of the study will be the identification of the various possible options, a second stage will be the calculation of the investment and operational maintenance costs for each of the options.

When these data have been collected it will be possible to determine and discuss the man-Sievert values associated with each of the protection options envisaged.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-F-423-81-F

sub-contract : SC-004-F

General subject of the contract:

Research to define the implicit values of an occupational health hazard : the case of vinyl-chloride-monomer.

Description of research work:

There are two possible approaches to the study of the cost of a radiological health hazard :
(a) determining a priori, with reference to an economic theory, "the cost of a human life",
(b) determining the cost a posteriori, by means of a retrospective analysis of earlier decisions relating to protection, leading to the establishment of the implicit values of human life : i.e. what has actually been spent on saving a theoretical, statistical human life in the nuclear and non-nuclear sectors.
The intended work comes under the second heading. Studies have either been carried out or are actually being carried out with regard to : (a) the implicit cost of avoided health effects occasioned by hazards in nuclear reactors, with respect to both protection of the general public against radiation and protection of the workers, (b) the implicit cost of avoided health effects occasioned by the occupational hazard in asbestos industries. This study has been completed in 1980.
Since this type of study is useful for the purposes of comparison, it is proposed to determine the costs of avoided health effects for the general public, for the industrial sector producing polymers and monomers of vinylchloride.
It has been established that exposure to low levels of monovinyl chloride (MVC) involves a cancer risk (angiosarcoma). For this reason a number of countries such as the USA, Canada and the Federal Republic of Germany have laid down regulations governing liquid and gaseous emissions of MVC and PVC (polyvinyl chloride). France is also planning to adopt regulations in this field.
It is therefore proposed to determine the cost of avoiding angiosarcoma in people living in the vicinity of these installations. The study will deal with atmospheric emissions which are the most important in factories producing MVC and PVC.
The various options leading to a reduction in discharges of MVC will be studied for these factories, together with an assessment of investment, and operational maintenance costs.
The second stage of the study will involve the assessment of the reduction in ambient concentrations, using a suitable atmospheric transfer model taking into account the prevailing meteorological conditions in the areas in which the factories are sited.

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Continuation contract no.: BIO-F-423-81-F ; sub-contract : SC-004-F

In the third stage the number of angiosarcomas avoided as a result of reduced ambient concentrations will be determined. The known relationships between dose and risk are mainly derived from studies on animals. The fact that the extrapolation of these relationships may lead to greater uncertainty than in the case of asbestos in particular, should not, however, be regarded as a major obstacle.
Since in the vast majority of cases no dose-risk relationship has actually been established, the question of uncertainty will be dealt with in the quantitative assessment of risks.
The comparison of the implicit costs computed in this study as well as in the previous ones (asbestos, nuclear reactors) will enable to discuss the value of the man-Sievert for the general public and for workers and, more generally, to compare the management of occupational and public risks in the various industrial sectors considered.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-F-423-81-F

sub-contract : SC-001-UK

General subject of the contract:

Assessment of collective doses from planned and unplanned releases to the atmosphere.

Description of research work:

The principal objective of the programme is the completion of the major task of combining into systematic, readable contract reports the achievements of the former five year contract period. Some continuing work will also be carried out on applying MESOS to unplanned releases; our original proposed objectives included an assessment of the potential of the MESOS techniques for application to short term unplanned releases. This aspect has evolved into a major item with the added stimulus from the Meteo Experts Group, and MESOS has been shown to be a powerful tool in assessing contamination out to longer distances in hypothetical accident situations. Overall the objectives for 1981 are :

1. To complete the plotting and presentation for printing of collective dose patterns for continuous (planned) releases from the 5 sites and the 2 data bases. Comparison of results using the 2 data bases, 1973 and 1976, will be completed for the Mol site.
2. To study the influence of the Alps and the Mediterranean region on trajectories arising from Ispra and Cadarache and the influence of this upon the annual average collective dose patterns.
3. To analyse cumulative probability distributions of contamination at particular locations arising from short (unplanned) releases, 3 hours to 7 days, for Ispra and Cadarache.
4. To extend the work of fitting parametric functions to cumulative probability distributions of contamination from unplanned releases, with the aim of making the large amount of data available in the future for wider practical use in safety assessment. Supplementary support from DG V Luxembourg will be needed to complete this special study.
5. To complete the work of preparing reports, for wider circulation, on the calculations relating to planned and unplanned releases and on the methods used.

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-F-423-81-F

sub-contract : SC-005-F

General subject of the contract:

The variability of individual doses as a factor in the assessment of the costs of the man-Sievert.

Description of research work:

In the ICRP's original formulation, the cost of the radiological hazard depended only upon the collective dose and did not take into account the distribution of individual doses. No distinction was made therefore between situations where, although the collective dose is the same :
(a) a very considerable number of people receive very low doses (e.g. 10.000 people each receiving 1 mrem),
(b) a very low number of people are subjected to far higher doses (e.g. 10 people each receiving 1 rem).
There are several possible approaches to deal with this difficulty. NRPB economists in the United Kingdom have proposed to make the value of α, the cost of one man-Sievert, variable according to the average individual doses received. Other experts are, however, considering the possibility of formulating the cost of the hazard as follows :

$$Y = \alpha S + \beta \sum N_i f(H_i)$$

where S is the collective dose, N_i the total number exposed and H_i the dose "per caput", α and β being constants.
It is intended to make a comparison of the various possible methodologies : variation of α, introduction of β, use of an utility function, as a follow-up to previous work.
This comparison will reveal :
(a) possible links between the various methods, the presuppositions associated with each of the latter : linearity without threshold of the dose-risk relationship or use of curvilinear dose-risk relationships (e.g. lineo-quadratic, quadratic).
(b) difficulties in applying some of these methods : for example, where the value of α is varied, account must be taken of the individual doses associated with all the other sources of irradiation (natural, medical, etc). On the basis of this comparison, an essentially pragmatic methodology will be proposed to take account of the variability of individual doses, without need, however, for detailed knowledge of all the types of irradiation (natural, medical, domestic, etc).

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RADIATION PROTECTION PROGRAMME

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Contract no.: BIO-F-423-81-F

sub-contract : SC-006-F

General subject of the contract:

Comparative analysis of diffusion and transfer models for releases in marine waters.

Description of research work:

The study is a part of the research to establish methodologies for the evaluation of the radiological impact of liquid effluent releases from nuclear power plants.

Effluent releases disperse in the marine environment under the combined effect of currents and the physical and physico-chemical mechanisms of sedimentation. Modelling of these phenomena has the dual objective of identifying the areas likely to be affected, and of assessing the pollutant concentration in the water. Modelling generally operates on two distinct, complementary levels. The "local" model is concerned only with an area restricted to a few kilometres from the point of release, and with the critical group of the population. At greater distances the "regional" model, which will be the main subject of the study, must integrate not only the movements of masses of water between the release area and more distant areas, but also exchanges with neighbouring seas; the population considered is regional, national or international. A number of solutions (of varying degrees of sophistication) have been proposed for either the local or regional model ; with regard to the regional model, for example, the joint study of CEA and NRPB (1979) favoured the theory of compartments, whereas oceanographers are more concerned with the mathematical formalization of currents which may contribute to forecasting of the route pollutants are likely to follow. The aim is to compare models of the dispersion of releases in seawater, the European seas being considered.

1. Initially a bibliographical study will be made and it will be based on :

a) the model and releases into seawater ;
b) the model and radioactive releases into seawater ;
c) the model and sea currents.

At this stage, attention will also be given to publications dealing with both radioactive releases into seawater and measured concentrations of radioelements in European seawater ; it is obviously necessary to be familiar with the concentration practices, but it is equally important to be able to evaluate their variability.

2. In the second stage, the published models will be analyzed. An attempt will be made to compare certain "typical" models representative of a method, and/or a geographical framework.

For a given radionuclide, an answer will be sought for the following questions :

- do the models provide comparable estimates for a unit amount released ?
- in the case of continuous releases, are the computed concentrations equivalent to those measured ?

For the purposes of this comparison, it will either be necessary to use published results computed with the models, or to use directly the computer codes referred to and available from the authors.

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RADIATION PROTECTION PROGRAMME

Head(s) of research teams(s):

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Contract no.: BIO-F-425-81-UK

General subject of the contract:

Development of fundamental data for radiation protection.

Description of research work:

In 1977 ICRP published a revised set of comprehensive basic recommendations. Since then ICRP has initiated a programme of work intended to elucidate the application of its current basic recommendations, and to revise former publications in the light of the new recommendations. In the period up to 1984 the Commission will continue its role of developing the fundamental data related to the establishment of appropriate recommendations on radiological protection. This will involve the study of the biological effects of radiation, including the short-term and late-developing effects (both somatic and hereditary) and the assessment of radionuclide behaviour in man and in his environment. The work outlined above will be part of ICRP's long-term programme designed for reviewing its recommendations for primary and secondary radiation standards.

II. APPENDIX

RESEARCH GROUP LEADERS

RESEARCH GROUP LEADERS

(continued)

RESEARCH GROUP LEADERS

(continued)

GEOGRAPHICAL DISTRIBUTION OF CONTRACTS

GEOGRAPHICAL DISTRIBUTION OF CONTRACTS

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GEOGRAPHICAL DISTRIBUTION OF CONTRACTS

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DISTRIBUTION OF CONTRACTS AMONG INSTITUTIONS

Country	Research Institutions	Universities	Others
Belgie/ Belgique	CEN/SCK 82,84,91, 105,185,198,243	Bruxelles 113, 134,260 Gent 78 Louvain 88,128, 234	
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