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EURATOM

Årsberetning 1974

PROGRAM BIOLOGI - SUNDHEDSBESKYTTELSE

Jahresbericht 1974

PROGRAMM BIOLOGIE - GESUNDHEITSSCHUTZ

Annual Report 1974

PROGRAMME BIOLOGY - HEALTH PROTECTION

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PROGRAMME BIOLOGIE - PROTECTION SANITAIRE

Relazione Annuale 1974

PROGRAMMA BIOLOGIA - PROTEZIONE SANITARIA

Jaarverslag 1974

PROGRAMMA BIOLOGIE - GEZONDHEIDSBESCHERMING

I

EUR 5332 d-e-f-i-n

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☐ 2. Genetiske virkninger af stråling.

☐ 3. Strålingsvirkninger på kort sigt, akut strålingssyndrom og dets behandling.

☐ 4. Strålingsvirkninger på langt sigt og inkorporerede radionukleiders toksikologi.

☐ 5. Strålingsmåling og dens fortolkning; dosimetri.

☐ 6. Anvendelse af strålingsbeskyttelsens, strålingsbiologiens og kerneteknikkens resultater inden for medicinsk forskning.

☐ 7. Anvendelse af strålingsbeskyttelsens, strålingsbiologiens og kerneteknikkens resultater inden for landbrugsforskning.

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☐ 2. Genetische Strahlenwirkungen.

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☐ 4. Spätwirkungen bei Bestrahlung und Toxikologie inkorporierter Radionuklide.

☐ 5. Strahlenmessung und ihre interpretation, Dosimetrie.

☐ 6. Anwendung der Ergebnisse aus Strahlenschutz, Strahlenbiologie und Kerntechnik in der medizinischen Forschung.

☐ 7. Anwendung der Ergebnisse aus Strahlenschutz, Strahlenbiologie und Kerntechnik in der landwirtschaftlichen Forschung.

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- ☐ 6. Application of the knowledge gained in radiation protection, radiobiology and nuclear techniques to medical research.
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- ☐ 4. Effets à long terme des rayonnements et toxicologie des radionuclides ingérés.
- ☐ 5. Mesures des rayonnements et leur interprétation, dosimétrie.
- ☐ 6. Application des connaissances acquises en radioprotection, radiobiologie et techniques nucléaires à la recherche médicale.
- ☐ 7. Application des connaissances acquises en radioprotection, radiobiologie et techniques nucléaires à la recherche agronomique.

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Directorate-general
for Research, Science and Education

Biology Division

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- ☐ 4. Effetti a lungo termine delle radiazioni e tossicologia dei radionuclidi incorporati.
- ☐ 5. Misura delle radiazioni e loro interpretazione, dosimetria.
- ☐ 6. Applicazione alla ricerca medica delle conoscenze acquisite in radioprotezione, radiobiologia e tecniche nucleari.
- ☐ 7. Applicazione alla ricerca agronomica delle conoscenze acquisite in radioprotezione, radiobiologia e tecniche nucleari.

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- ☐ 3. Effecten van straling op korte termijn, acuut bestralingssyndroom en behandeling.
- ☐ 4. Effecten van straling op langer termijn en toxicologie van opgenomen radionucliden.
- ☐ 5. Meting van straling en de interpretatie daarvan, dosimetrie.
- ☐ 6. Toepassing van de verworven kennis op het gebied van stralingsbescherming, stralingsbiologie en kerntechniek bij medisch onderzoek.
- ☐ 7. Toepassing van de verworven kennis op het gebied van stralingsbescherming, stralingsbiologie en kerntechniek bij landbouwkundig onderzoek.

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The annual reports in this volume were prepared under the responsibility of the heads of the research teams, set up under the various contracts, and were submitted in this form to the Commission and its contractual partners.

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INDLEDNING

Dk

Tre staters tiltrædelse af De europæiske Fællesskaber den 1. januar 1973 har gjort det nødvendigt at tilpasse programmet "Biologi - sundhedsbeskyttelse" til den nye situation, der hermed er opstået. Afsnittet "Strålingsbeskyttelse" blev tilpasset ved en afgørelse i Ministerrådet den 14. maj 1973, og kontrakter med britiske, danske og irske organer trådte i kraft i 1974. Følgelig indeholder dette bind for første gang rapporter over de fremskridt, der er gjort i de nye medlemsstater, og konkretiserer dermed den gradvise integrering af forskningen, som blev foreslået af Kommissionen i dennes udkast til en tilpasning af programmet.

For så vidt angår afsnittet "Anvendelser", et supplerende program med Tyskland, Italien og Nederlandene som deltagere, blev dette tilpasset af Ministerrådet den 2. august 1974, som besluttede, at Danmark og Irland skulle deltage i programmet samtidig med de tre oprindeligt deltagende stater.

Det må ligeledes bemærkes, at en aktiv forberedelse af et udkast til forslag om et nyt program for 1976-1980 har fundet sted i løbet af anden halvdel af 1974. Adskillige studiegrupper er mødtes og har for programmets forskellige forskningsemner foretaget en undersøgelse af den nuværende viden, de fremtidige behov og orienteringen af det fremtidige arbejde. I denne sammenhæng har der fundet en første udveksling af synspunkter sted vedrørende de store linier i et fremtidigt program under det møde, som i december 1974 afholdtes i Det rådgivende udvalg for Programforvaltning "Biologi - sundhedsbeskyttelse". (Det blev meget frugtbringende for Kommissionens tjenestegrene, for hvilke det blev muligt at tage et vigtigt skridt fremad med hensyn til forberedelsen af forslagsudkastet).

For så vidt angår selve programmet er det værd at erindre, at det består af et afsnit benævnt "Strålingsbeskyttelse" (fællesprogram) og et afsnit benævnt "anvendelser" (supplerende program), hvis forskningsområder og hvis mål kan skitseres som følger:

1. Måling og vurdering af den ioniserende stråling, som mennesket og forskellige dele af omgivelserne udsættes for:

   - dosimetri, strålingsmåling og fortolkning af måleresultaterne;
   - undersøgelse af transporten og ophobningen af radionukleider i mennesket og i omgivelsernes bestanddele.

2. De ioniserende strålers vekselvirkning med de biologiske systemer:

   - strålingens primærvirkninger
   - virkninger på generne
   - kortsigtede virkninger
   - langsigtede virkninger.

3. Anvendelser af kerneteknik inden for visse af den landbrugsvidenskabelige og medicinske forsknings vigtigste områder.

Dette dokument indeholder "fremskridtsrapporterne" for kontraktprogrammets enkeltprojekter og for gruppen Biologi i Ispra.

F. VAN HOECK P. RECHT

D

EINLEITUNG

Durch den Beitritt der drei neuen Mitgliedstaaten zu den Europäischen Gemeinschaften am 1. Januar 1973 wurde es notwendig, das Programm "Biologie - Gesundheitsschutz" der hierdurch entstandenen neuen Situation anzupassen. Der Sektor "Strahlenschutz" wurde durch einen Ministerratsbeschluß vom 14. Mai 1973 geändert, und die Verträge mit den britischen, dänischen und irischen Institutionen traten 1974 in Kraft. Der vorliegende Jahresbericht enthält erstmals Forschungsergebnisse aus den neuen Mitgliedstaaten; die schrittweise Integrierung der Forschung, die die Kommission in ihrem Entwurf zur Anpassung des Programms vorgeschlagen hatte, nimmt damit konkrete Form an.

Der Sektor "Anwendungen", ein Ergänzungsprogramm, an dem die Bundesrepublik Deutschland, Italien und die Niederlande beteiligt sind, wurde vom Ministerrat am 2. August 1974 angepaßt, mit der Maßgabe, daß Dänemark und Irland zusammen mit den drei ursprünglichen Mitgliedstaaten teilnehmen.

Zu erwähnen ist ferner die Vorbereitung des Entwurfs eines neuen Forschungsprogramms für die Jahre 1976-1980 während der zweiten Hälfte 1974. Mehrere Studiengruppen trafen sich und untersuchten für die einzelnen Themen und Punkte des Forschungsprogramms den Stand der Kenntnisse, den künftigen Bedarf und welche Leitlinien für die Zukunft zu verfolgen seien. Desgleichen fand auf der Sitzung des Beratenden Programmausschusses "Biologie - Gesundheitsschutz" vom Dezember 1974 ein erster Gedankenaustausch über die Schwerpunkte des zukünftigen Programms statt. Die Dienststellen der Kommission gewannen daraus Anregung und Unterstützung für die weiterzuführende Vorbereitung des Programmvorschlages.

Was das Programm selbst betrifft, so sei bemerkt, daß es einen Abschnitt "Strahlenschutz" (gemeinsames Programm) und einen Abschnitt "Anwendungen" (Ergänzungsprogramm) umfaßt, deren Forschungsbereiche und Ziele nach folgendem Schema dargestellt werden können:

1. Messung und Bewertung der Belastung des Menschen und seiner Umwelt durch ionisierende Strahlungen:
   - Dosimetrie, Strahlenmessung und ihre Interpretation;
   - Untersuchung des Transports und der Anreicherung der Radionuklide im Menschen und in seiner Umwelt.

2. Wechselwirkung der ionisierenden Strahlungen mit den biologischen Systemen:
   - Primärwirkungen der Strahlungen
   - Wirkungen auf das Erbgut
   - Kurzzeitwirkungen
   - Langzeitwirkungen.

3. Anwendungen der nuklearen Techniken auf bestimmte wichtige Sektoren der agronomischen und medizinischen Forschung.

Das vorliegende Dokument enthält die "Berichte über den Fortgang der Arbeiten" an den einzelnen Projekten des Vertragsprogramms und die Berichte der Gruppe Biologie Ispra.

F. VAN HOECK P. RECHT

INTRODUCTION

The accession of three States to the European Communities on 1 January 1973 made it necessary to adapt the programme "Biology - Health Protection" to the new situation which thus arose. The "Radiation Protection" part was adjusted by a decision of the Council of Ministers of 14 May 1973, and contracts with British, Danish and Irish bodies came into effect in 1974. As a result, this volume presents for the first time progress reports from the new Member States, setting out in concrete form the progressive integration of research which had been proposed by the Commission in its draft programme adjustment.

The "Applications" part, a supplementary programme in which the Federal Republic of Germany, Italy and the Netherlands participated, was adjusted by the Council of Ministers on 2 August 1974, which decided that Denmark and Ireland would participate at the same time as the three original Member States.

It must also be indicated that the active preparation of a draft proposal for a new 1976-1980 programme was effected during the latter half of 1974. Several study groups met and studied, with regard to the various research themes and subjects of the programme, the state of the art, future requirements, and the guidelines to be adopted for the future. In the same context, an initial discussion on the broad outline of a future programme was held during the meeting of the Advisory Committee on Programme Management "Biology - Health Protection" in December 1974. This discussion turned out to be most rewarding for the departments of the Commission concerned, whom it enabled to take an important step in the preparation of the draft proposal.

With regard to the programme itself, it should be mentioned that it includes a "Radiation Protection" (common programme) sector and an "Applications" (supplementary programme) sector whose fields of research and objectives can be outlined as follows:

1. Measurement and evaluation of the exposure of man and the various components of the ambient environment to ionizing radiation:

   - dosimetry, radiation measurements and their interpretation;
   - study of the transfer and accumulation of radionuclides in man and in the constituents of the environment.

2. Interaction of ionizing radiations with biological systems:

   - primary effects of radiation;
   - effects on hereditary material;
   - short-term effects;
   - long-term effects.

3. Application of nuclear techniques in certain important sectors of agricultural and medical research.

This document presents the "progress reports" for each project of the contractual programme and of the Biology Group Ispra.

F. VAN HOECK P. RECHT

F

INTRODUCTION

L'adhésion de trois Etats aux Communautés européennes, le 1er janvier 1973, a rendu nécessaire l'adaptation du programme "Biologie - Protection sanitaire" à la nouvelle situation ainsi créée. Le secteur "Radioprotection" fut aménagé par une décision du Conseil de Ministres du 14 mai 1973, et des contrats avec des organismes britanniques, danois et irlandais prirent cours en 1974. Par conséquent, le présent volume présente pour la première fois des rapports d'avancement en provenance des nouveaux Etats membres, concrétisant l'intégration progressive des recherches qui avait été proposée par la Commission dans son projet d'aménagement du programme.

Quant au secteur "Applications", programme complémentaire auquel participaient l'Allemagne, l'Italie et les Pays-Bas, il fut aménagé par le Conseil de Ministres le 2 août 1974, qui décida que le Danemark et l'Irlande participeraient au programme en même temps que les trois anciens Etats participants.

Il convient également de signaler qu'une préparation active d'un projet de proposition pour un nouveau programme 1976-1980 a eu lieu au cours de la deuxième moitié de 1974. Plusieurs groupes d'études se sont réunis et ont étudié, pour les divers thèmes et sujets de recherche du programme, l'état des connaissances, les besoins futurs, et les orientations à adopter pour l'avenir. Dans la même optique, un premier échange de vue sur les grandes lignes d'un futur programme a eu lieu au cours de la réunion du Comité consultatif en matière de gestion de programmes "Biologie - Protection sanitaire" de décembre 1974. Il fut très fructueux pour les services de la Commission, auxquels il permit de franchir un pas important dans la préparation du projet de proposition.

En ce qui concerne le programme lui-même, il convient de rappeler qu'il comporte un secteur "Radioprotection" (programme commun) et un secteur "Applications" (programme complémentaire) dont les domaines de recherche et les objectifs peuvent être schématisés comme suit:

1. Mesure et évaluation de l'exposition de l'homme et des diverses composantes du milieu ambiant aux rayonnements ionisants:
   - dosimétrie, mesure des rayonnements et leur interprétation;
   - étude du transfert et de l'accumulation des radionucléides dans l'homme et dans les éléments du milieu.

2. Interaction des rayonnements ionisants avec les systèmes biologiques:
   - effets primaires des rayonnements
   - effets sur le matériel héréditaire
   - effets à court terme
   - effets à long terme.

3. Applications des techniques nucléaires à certains secteurs importants de la recherche agronomique et médicale.

Le présent document présente les "rapports d'avancement" par projet individuel du programme contractuel et du groupe de Biologie installé à Ispra.

F. VAN HOECK P. RECHT

INTRODUZIONE

L'adesione di tre Stati alle Comunità europee, avvenuta il 1° gennaio 1973, ha reso necessario procedere ad un adeguamento del programma "Biologia-Protezione sanitaria" alla nuova situazione creatasi. Il settore "Radioprotezione" è stato riorganizzato con la decisione del Consiglio dei Ministri del 14 maggio 1973 e nel 1974 sono entrati in vigore contratti con organismi britannici, danesi e irlandesi. Pertanto, questo volume presenta per la prima volta delle relazioni di avanzamento dei lavori, provenienti dai nuovi Stati membri, dando forma concreta al progressivo inglobamento delle ricerche come proposte dalla Commissione nel suo progetto di riorganizzazione del programma.

Per quanto riguarda il settore "Applicazioni", il programma complementare al quale partecipavano la Germania, l'Italia e i Paesi Bassi, è stato rimaneggiato dal Consiglio dei Ministri il 2 agosto 1974 con una decisione che prevedeva la partecipazione della Danimarca e dell'Irlanda al programma a fianco dei tre Stati membri precitati.

Occorre segnalare inoltre che durante la seconda metà del 1974 ha avuto luogo una preparazione attiva di un progetto di proposta per un nuovo programma 1976-1980. Vari gruppi di studio si sono riuniti per esaminare per i diversi temi e soggetti di ricerca del programma lo stato delle conoscenze, i fabbisogni futuri e gli orientamenti da seguire in avvenire. Attenendosi allo stesso punto di vista, nel corso della riunione del dicembre 1974 del Comitato consultivo in materia di gestione dei programmi "Biologia-Protezione sanitaria", ha avuto luogo un primo scambio di vedute circa le grandi linee direttrici di un programma futuro. Tale riunione è stata molto utile ai servizi della Commissione, che hanno potuto compiere un grande passo avanti nella preparazione delle proposte.

Circa il programma, si ricorda che esso comporta una sezione "Radioprotezione" (programma comune) e una sezione "Applicazioni" (programma complementare), i cui settori di ricerca e gli obiettivi possono essere schematizzati nel modo seguente:

1. misura e valutazione dell'esposizione dell'uomo e dei vari componenti dell'ambiente alle radiazioni ionizzanti:
   - dosimetria, misura delle radiazioni e interpretazione dei risultati;
   - studio del passaggio e dell'accumulazione dei radionuclidi nell'uomo e negli elementi dell'ambiente;

2. interazione delle radiazioni ionizzanti con i sistemi biologici:
   - effetti primari delle radiazioni
   - effetti sul materiale ereditario
   - effetti a breve termine
   - effetti a lungo termine;

3. applicazione delle tecniche nucleari in alcuni importanti settori della ricerca agronomica e medica.

Il presente documento contiene le relazioni sull'avanzamento dei singoli progetti del programma contrattuale e del gruppo biologia con sede ad Ispra.

F. VAN HOECK P. RECHT

INLEIDING

Door de toetreding van de drie staten tot de Europese Gemeenschappen op 1 januari 1973 moest het programma "Biologie - bescherming van de gezondheid" aan de nieuwe situatie worden aangepast. De sector Stralingsbescherming werd opnieuw geordend door een besluit van de Raad van Ministers van 14 mei 1973, en contracten met Britse, Deense en Ierse organisaties begonnen in 1974 te lopen.
Bijgevolg omvat dit volume voor de eerste maal rapporten over de vordering van het onderzoek, die afkomstig zijn van de nieuwe lid-staten, en waarin de geleidelijke integratie wordt geconcretiseerd van de onderzoekingen, die door de Commissie in haar ontwerp tot wijziging van het programma werd voorgesteld.

De sector Toepassingen, een aanvullend programma waaraan Duitsland, Italië en Nederland deelnamen, werd op 2 augustus 1974 opnieuw door de Raad van Ministers geordend : de Raad besloot dat Denemarken en Ierland tegelijkertijd met de drie al eerder deelnemende staten aan het programma zouden deelnemen.

Er moet tevens worden vermeld dat tijdens de tweede helft van 1974 het ontwerp-voorstel voor een nieuw programma 1976-1980 intensief werd voorbereid. Een aantal studiegroepen zijn bijeengekomen en hebben voor de verschillende thema's en onderzoekonderwerpen van het programma een studie gemaakt over de stand van de kennis, de toekomstige behoeften, en de richtlijnen die voor de toekomst moeten worden goedgekeurd. Uit hetzelfde oogpunt, heeft tijdens de vergadering van het raadgevend comité op het gebied van het programmabeheer "Biologie - bescherming van de gezondheid" van december 1974 een gedachtenwisseling plaatsgevonden over de grote lijnen van een toekomstig programma. Deze gedachtenwisseling was heel nuttig voor de diensten van de Commissie die hierdoor een belangrijke stap vooruit konden zetten bij de voorbereiding van het ontwerp-voorstel.

Wat het programma zelf betreft, moet erop gewezen worden dat dit een sector Stralingsbescherming (gemeenschappelijk programma) omvat en een sector Toepassingen (aanvullend programma), waarvan de gebieden en doelstellingen als volgt in een schema kunnen worden samengevat :

1. Meting en evaluatie van de blootstelling van de mens en verschillende componenten van het omgevingsmilieu aan ioniserende straling :
   - dosismeting, stralingsmeting en interpretatie hiervan;
   - studie van de overdracht en accumulatie van de radionucliden bij de mens en bij de milieucomponenten.

2. Interactie van de ioniserende stralingen met de biologische systemen :
   - primaire effecten van stralingen
   - effecten op het erfelijk materiaal
   - effecten op korte termijn
   - effecten op lange termijn.

3. Toepassingen van de nucleaire technieken op bepaalde belangrijke sectoren van het agronomisch en medisch onderzoek.

Dit document omvat de "rapporten over de vordering van het onderzoek" per individueel project van het contractueel programma en van de groep Biologie te Ispra.

F. VAN HOECK P. RECHT

Mitglieder im Jahr 1974 des Beratenden Programmausschusses
"BIOLOGIE - GESUNDHEITSSCHUTZ"

Members in 1974 of the Advisory Committee on Programme Management
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III.

FORSCHUNGSTÄTIGKEIT STRAHLENSCHUTZ

RESEARCH IN RADIATION PROTECTION

RECHERCHES EN RADIOPROTECTION

STRAHLENMESSUNGEN UND IHRE INTERPRETATION (DOSIMETRIE)

MEASUREMENT AND INTERPRETATION OF RADIATION (DOSIMETRY)

MESURE DES RAYONNEMENTS ET LEUR INTERPRETATION (DOSIMETRIE)

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

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

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

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

Biology Group Ispra

Radiobiological Institute TNO, Rijswijk (ZH), The Netherlands

Contract No. 101-72-1 BIOC

G.W. Barendsen

Evaluation of the biological effectiveness of different types of radiation

Energy deposition spectra measured with tissue-equivalent proportional counters, can provide basic information for the prediction of the relative biological effectiveness of ionizing radiations for various effects on cells. The cylindrical tissue-equivalent proportional counter used for the present measurements has an elongation factor of 10 and consequently, energy deposition spectra could be expected to depend on the angle between the counter axis and beam direction. This effect has been evaluated for 15 MeV neutrons, and it was demonstrated that significant variations can be observed for the fast proton component.

With respect to the application of fast neutrons in radiotherapy, it is important to know whether due to the collimators used the energy deposition spectra are influenced and whether they vary with the distance from the beam axis. Measurements have demonstrated that significant variations occur which might cause a variation of the RBE.

Results of project No. 1

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

Evaluation of the biological effectiveness of different types of radiation

Evaluation of the biological effectiveness of different types of radiation from measurements of energy deposition spectra for alpha particles as a function of energy and of corresponding survival curves, and assuming the single step action model for the loss of reproductive capacity of mammalian cells after irradiation, it was concluded earlier that the critical structure in cells might have dimensions equivalent to 10-100 nm of unit density tissue.

For the measurement of event size distributions in small volumes the best detector at present available is provided by the tissue-equivalent (TE) proportional counter. From earlier measurements of event size distributions for alpha particles passing through the sensitive volume of the cylindrical TE-counter over two trajectories of different length, it was concluded that the smallest diameter, which can be simulated by the counter, is equivalent to 0.15 um of unit density tissue. Measurements of event size distributions for neutrons have now demonstrated that at this simulated dimension the spectra are distorted and that only diameters down to about 0.25 μm can be simulated without spectrum distortion. Thus further measurements have been confined to simulate only volumes in excess of 0.25 μm in diameter.

In order to evaluate in further detail whether the shape of the counter volume can influence the spectra, energy deposition spectra have been measured for 3 and 15 MeV neutrons with a TE-counter, simulating cylindrical tissue-equivalent volumes of various diameters and elongation factor of 10, at angles of incidence of the neutron beam of 0, 30, 60 and 75 degrees, respectively.

The spectra for 15 MeV neutrons at an equivalent diameter of 5.6 um unit density are presented in figure 1. The position of peak in these spectra in the region of 1-10 keV/μm originating from energy deposition by fast protons, shows a dependence on the angle of incidence, which is inversely proportional to the cosinus of this angle.

In the application of neutron sources for therapeutical purpose, collimators and shielding have to be applied in order to produce narrow well-defined beams. Due to scatter and absorption in this shielding and collimator material, the spectrum of neutrons in a patient will not be identical to the face in air

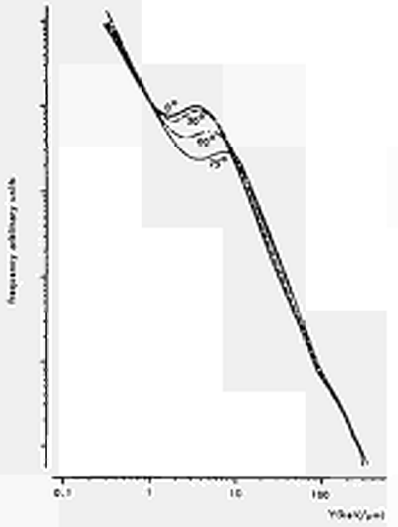


Figure 1. Event size spectra of 15 MeV neutrons measured with a cylindrical counter for various angles of incidence of the neutron beam; simulated diameter of the volume is 5.6 µm of unit density tissue.

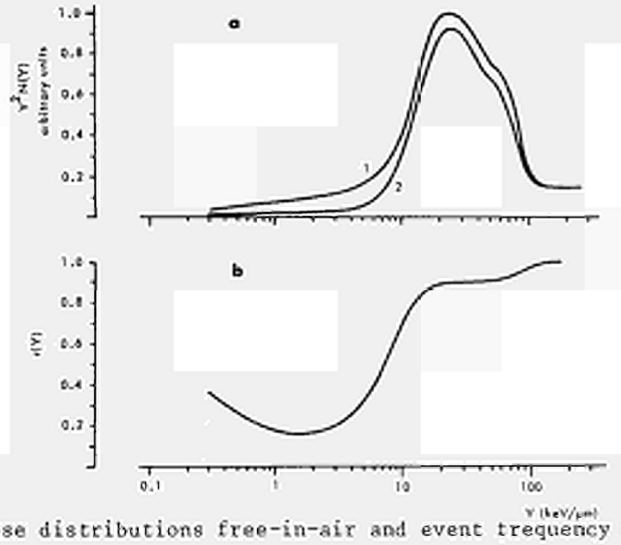


Figure 2. Dose distributions free-in-air and event frequency ratio curve of 3 MeV neutrons with collimator.

a: Measured dose distributions $Y^2N(Y)$ of collimated 3 MeV neutron beams for equal neutron fluxes (Y = event size; N(Y) = event size distribution).

b: Ratio curve $r(Y) = N_2(Y)/N_1(Y)$ for the measured spectra of a. The indices refer to positions as mentioned in a.

spectrum of the source. Consequently, the radiation quality may change over the irradiated region.

Energy deposition distributions for collimated beams of 3 and 15 MeV have been measured at different positions in the collimated beam with and without phantom. The distributions at the centre and at the edge of the beam, without phantom, for 3 MeV neutrons is presented in figure 2. These results show, that the contribution of low energy events at the edge is smaller than at the centre. Consequently, a lower RBE value at the centre as compared to the edge position can be expected. For 15 MeV neutrons the variations in event size distributions as a function of the distance from the centre of the beam are much smaller. This indicates that these high energy monoenergetic neutrons do not present specific problems with respect to therapeutical applications.

Measurements of event size distributions for lower neutron energies and in collimated beams will be the subject for further investigations.

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

Barendsen, G.W., Relative biological effectiveness and biological complexity. In: Proceedings of 4th Symp. Microdosimetry, Verbania Pallanza, Italy, 1973, Commission of the European Communities, Luxembourg, pp. 235-252 (1974).

Hogeweg, B., Gas gain characteristics of a tissue-equivalent proportional counter, and their implications for measurements of event size distributions in small volumes. Ibid, pp. 843-851.

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

N° du contrat : 101-72-1-BIOC

Chef du groupe de recherche : D. BLANC

Thème général du contrat : Energy transfer in biological material and in model substances.

Description générale succincte des travaux réalisés :

We have pursued our research in two main directions :

1) Transport simulation of low energy electrons in matter. At present we can simulate electron transport from 1 keV down to 20 eV in molecular media such as N_2, O_2, Air and calculate all the dosimetric and microdosimetric quantities.

2) Transport simulation of radiotherapy electrons beam in a medium irradiated through a diffusing screen : we simulated the electron transport into the screen and then into homogeneous tissue situated beyond.

Résultat du projet n° 1.
Chef du projet et collaborateurs scientifiques :
J. P. PATAU, M. TERRISSOL, J. FOURMENTY, J. P. MANNENS
Titre du projet : Simulation du transport des particules dans la matière par méthode de Monte-Carlo. Application à la dosimétrie.

Description des résultats

Des recherches se sont développées dans deux directions principales : 1) la simulation du transport des électrons de basse énergie (< 1 keV) dans la matière, 2) la simulation du transport d'un faisceau d'électrons de radiothérapie dans un milieu irradié à travers un cache diffuseur.

Le premier thème de recherche a fait l'objet de notre dernière publication au quatrième Symposium de Microdosimétrie de PALLANZA (1). Depuis nos efforts ont abouti à la simulation du transport des électrons de basse énergie dans des milieux gazeux diatomiques (O_2, N_2, air). Pour les collisions élastiques nous utilisons les sections efficaces différentielle et totale non relativiste dite "des déphasages de MOTT". Comme il s'agit de sections efficaces atomiques nous avons utilisé l'approximation des centres diffuseurs indépendants (2), afin de pouvoir simuler la diffusion élastique sur les molécules. Pour les intéractions inélastiques nous n'utilisons plus les sections efficaces totales théoriques de GRYZINSKI mais les résultats expérimentaux connus et exploitables, rassemblés par KIEFFER (3). Ces résultats sont introduits sur ordinateur sous forme analytique. Les efficacités des méthodes d'échantillonnages des sections efficaces différentielles polaires et de perte d'énergie de VRIENS ont été fortement améliorées par la méthode de composition.

Un programme général de simulation du transport des électrons a été mis au point et permet d'obtenir les distributions spatiales des points d'ionisation et de divers types d'excitation, ainsi que les distributions spatiales des dépôts d'énergie par ces deux processus. On peut en déduire toutes les quantités dosimétriques et microdosimétriques désirées (voir par exemple la courbe 1).

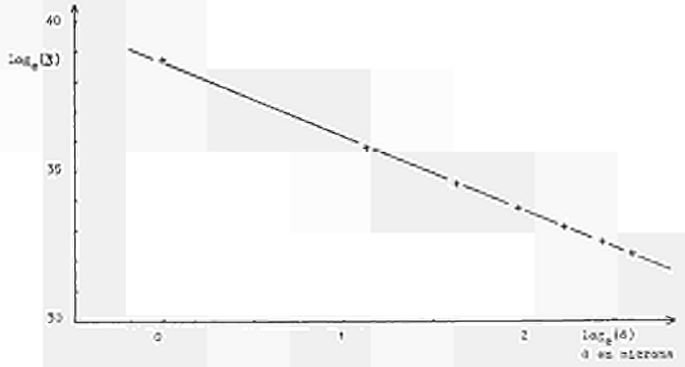
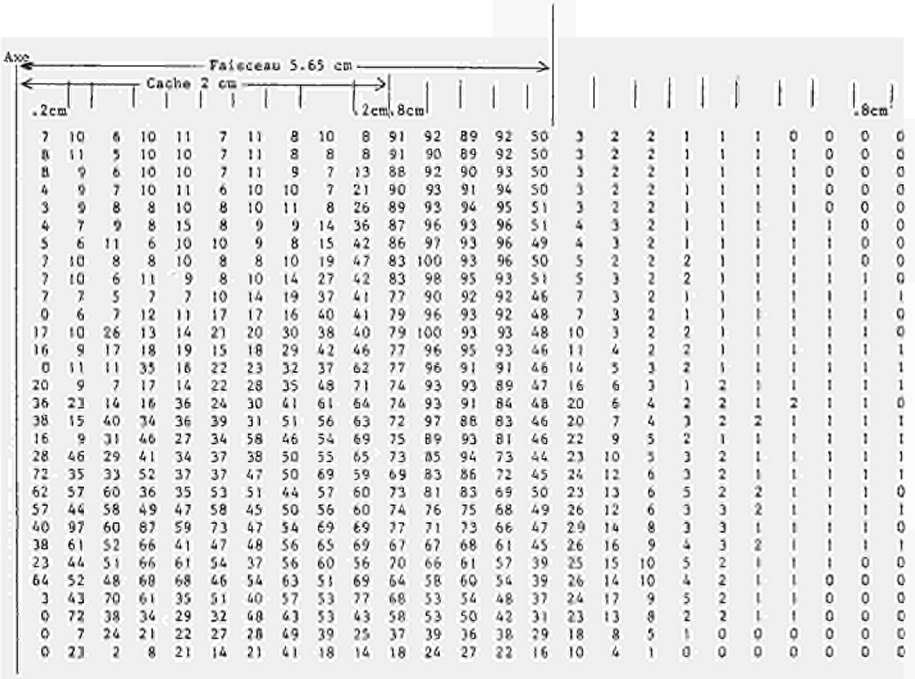


FIGURE 1 : Variations de $\mathcal{S}$ en eV/(g.el) en fonction du diamètre d pour un volume sphérique d'air situé dans l'air. Energie des électrons incidents 1 keV.



7	10	6	10	11	7	11	8	10	8	91	92	89	92	50	3	2	2	1	1	1	0	0	0	0
8	11	5	10	10	7	11	8	8	8	91	90	89	92	50	3	2	2	1	1	1	1	0	0	0
8	9	6	10	10	7	11	9	7	13	88	92	90	93	50	3	2	2	1	1	1	1	0	0	0
4	9	7	10	11	6	10	10	7	21	90	93	91	94	50	3	2	2	1	1	1	1	0	0	0
3	9	8	8	10	8	10	11	8	26	89	93	94	95	51	3	2	2	1	1	1	1	0	0	0
4	7	9	8	15	8	9	9	14	36	87	96	93	96	51	4	3	2	1	1	1	1	1	0	0
5	6	11	6	10	10	9	8	15	42	86	97	93	96	49	4	3	2	1	1	1	1	1	0	0
7	10	8	8	10	8	8	10	19	47	83	100	93	96	50	5	2	2	2	1	1	1	1	0	0
7	10	6	11	9	8	10	14	27	42	83	98	95	93	51	5	3	2	2	1	1	1	1	1	0
7	7	5	7	7	10	14	19	37	41	77	90	92	92	46	7	3	2	1	1	1	1	1	1	1
0	6	7	12	11	17	17	16	40	41	79	96	93	92	48	7	3	2	1	1	1	1	1	1	0
17	10	26	13	14	21	20	30	38	40	79	100	93	93	48	10	3	2	2	1	1	1	1	1	0
16	9	17	18	19	15	18	29	42	46	77	96	95	93	46	11	4	2	2	1	1	1	1	1	1
0	11	11	35	18	22	23	32	37	62	77	96	91	91	46	14	5	3	2	1	1	1	1	1	1
20	9	7	17	14	22	28	35	48	71	74	93	93	89	47	16	6	3	1	2	1	1	1	1	1
36	23	14	16	36	24	30	41	61	64	74	93	91	84	48	20	6	4	2	2	1	2	1	1	0
38	15	40	34	36	39	31	51	56	63	72	97	88	83	46	20	7	4	3	2	2	1	1	1	1
16	9	31	46	27	34	58	46	54	69	75	89	93	81	46	22	9	5	2	1	1	1	1	1	1
28	46	29	41	34	37	38	50	55	65	73	85	94	73	44	23	10	5	3	2	1	1	1	1	1
72	35	33	52	37	37	47	50	69	59	69	83	86	72	45	24	12	6	3	2	1	1	1	1	1
62	57	60	36	35	53	51	44	57	60	73	81	83	69	50	23	13	6	5	2	2	1	1	1	0
57	44	58	49	47	58	45	50	56	60	74	76	75	68	49	26	12	6	3	3	2	1	1	1	1
40	97	60	87	59	73	47	54	69	69	77	71	73	66	47	29	14	8	3	3	1	1	1	1	0
38	61	52	66	41	47	48	56	65	69	67	67	68	61	45	26	16	9	4	3	2	1	1	1	1
23	44	51	66	61	54	37	56	60	56	70	66	61	57	39	25	15	10	5	2	1	1	1	0	0
64	52	48	68	68	46	54	63	51	69	64	58	60	54	39	26	14	10	4	2	1	1	0	0	0
3	43	70	61	35	51	40	57	53	77	68	53	54	48	37	24	17	9	5	2	1	1	0	0	0
0	72	38	34	29	32	48	43	53	43	58	53	50	42	31	23	13	8	2	2	1	1	0	0	0
0	7	24	21	22	27	28	49	39	25	37	39	36	38	29	18	8	5	1	0	0	0	0	0	0
0	23	2	8	21	14	21	41	18	14	18	24	27	22	16	10	4	1	0	0	0	0	0	0	0

FIGURE 2 : Distribution spatiale d'énergie massique déposée. Cache en plomb. Energie des électrons incidents 28 MeV. Distance cache-tissu 25 cm.

Le second thème de recherche concerne un problème de radiothérapie. En effet, il est parfois nécessaire de traiter par ce procédé des tumeurs situées dans le voisinage d'un organe sain (cas des tumeurs de la région oculaire par exemple). Nous avons donc, à l'instigation de Madame A. DUTREIX, effectué la simulation du transport des électrons dans des caches diffuseurs ainsi que dans un milieu homogène de tissu mou supposé situé derrière.

Ces caches ne sont destinés qu'à diffuser le faisceau et non à l'arrêter ; il se présentent sous la forme de cylindres de faible épaisseur, 1/10ème à 1/5ème du parcours maximal des électrons primaires dans le milieu constitutif du cache ; leur diamètre varie de 2 à 4 cm. Ils sont constitués par du cadmium ou du plomb. Les faisceaux à la sortie de l'accélérateur ont des énergies de 28 et 10 MeV. Une étude systématique en fonction de tous les paramètres en cause a été effectuée. Les résultats seront publiés au symposium sur les progrès de la dosimétrie en biomédecine organisé à VIENNE (Autriche) par l'A. I. E. A. du 10 au 14 Mars 1975. La figure 2 montre un exemple de résultats obtenus.

REFERENCES

1) TERRISSOL (M.), PATAU (J. P.)
Proceedings Fourth Symposium on microdosimetry PALLANZA (Octobre 1973). J. BOOZ and H. G. EBERT editors. EUR S22 d-e-f.

2) MASSEY (H. S. W.)
Electronic and ionic impact phenomena. Vol. 2, page 668, Oxford University Press (1969).

3) KIEFFER (L. J.)
JILA information center report n° 13 (September 1973).

Vertragspartner der Kommission : Gesellschaft
für Strahlen- und Umweltforschung, München

Nr. des Vertrages : 101 - 72 - 1 BIO C

Leiter der Forschungsgruppen : Prof.Dr.W.Pohlit

Allgemeines Thema des Vertrages:
Dosimetrie in der Mikrobiologie

It is supposed, that in living cells there are indirect as well direct radiation effects. Their relative contributions to the inactivation of cells depend mainly on the linear energy transfer of the radiation used and on the environmental conditions of the targets within the irradiated cells.

For studies about the indirect radiation effect it is suitable to use scarcely ionizing radiations(e.g. ^{60}Co-gamma-radiation, fast electrons), since the G-values for the radicals $OH^{\bullet}$, e^-_{aq} and $H^{\bullet}$ are highest for these radiations. Furthermore, indirect radiation effects should be investigated in systems, in which direct radiation effects don't occur. Appropriate systems are dilute aqueous solutions of thymine. In such systems the destruction of the thymine chromophore by the radicals $OH^{\bullet}$, e^-_{aq}, $H^{\bullet}$ and $HO_2^{\bullet}$ was investigated quantitatively. By bubbling the solutions with different gases (N_2O, CO_2, N_2, O_2) and/or by addition of specific radical scavengers it was possible to determine the effectiveness of the radicals to destroy thymine chromophores. The main results are :

1. Each $OH^{\bullet}$ destroys a thymine chromophore.
2. Only 40 percent of all generated e^-_{aq} destroy thymine chromophores.
3. $H^{\bullet}$ and $HO_2^{\bullet}$ don't destroy thymine chromophores.
4. Oxygen has a small protective effect.
5. The anions SCN^- and $HCOO^-$ protect thymine chromophores against $OH^{\bullet}$.

Ergebnisse des Projekts Nr. II

Leiter des Projekts und wissenschaftliche Mitarbeiter: Dr. D. Frankenberg

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

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In all experiments the destruction of the thymine chromophore was measured following the decrease of absorbance, $\varepsilon_o - \varepsilon$, at λ = 265 nm as a function of the absorbed dose, D. The irradiations were performed with 30 MeV electrons, the LET-spectrum of which differ from that of ^{60}Co-gamma-radiation only in the very low LET-region ($L_{100} \leq 1$ keV/μm). Therefore the radical yields for 30 MeV electrons are the same as for ^{60}Co-gamma-radiation ($G_{OH\cdot} = 2.75(100eV)^{-1}$, $G_{e^-_{aq}} = 2.71(100eV)^{-1}$, $G_{H\cdot} = 0.58(100eV)^{-1}$). From the initial slopes of the dose effect curves in figure 1 the G-values for the destruction of thymine chromophores under various conditions i, $G_i(T^*)$, were obtained and are shown in table 1.

Using the conditions i=1 and i=2 only OH· and H· are present in the solution, since NO_3^- is an effective scavenger for e^-_{aq} and in strong acid solutions all e^-_{aq} are quantitatively converted into H· respectively. While the G-value for OH· is equal in both cases, the G-value for H· under condition i=2 is larger by a factor 5 compared to that under condition i=1. Since $G_1(T^*)$ and $G_2(T^*)$ are equal, it can be concluded, that each OH· destroys a thymine chromophore, whereas H·-radicals are ineffective in this respect. Furthermore, it was found, that the radicals $HO_2^\cdot$ and $\cdot CO_2^-$ don't destroy thymine chromophores, since $G_4(T^*)$ and $G_3(T^*)$ are equal to $G_1(T^*)$.

In the presence of N_2O the e^-_{aq} are quantitatively converted into OH·-radicals. Therefore, the difference $G_5(T^*)-G_1(T^*) = 2.69(100eV)^{-1}$ represents the G-value for e^-_{aq}. On the other

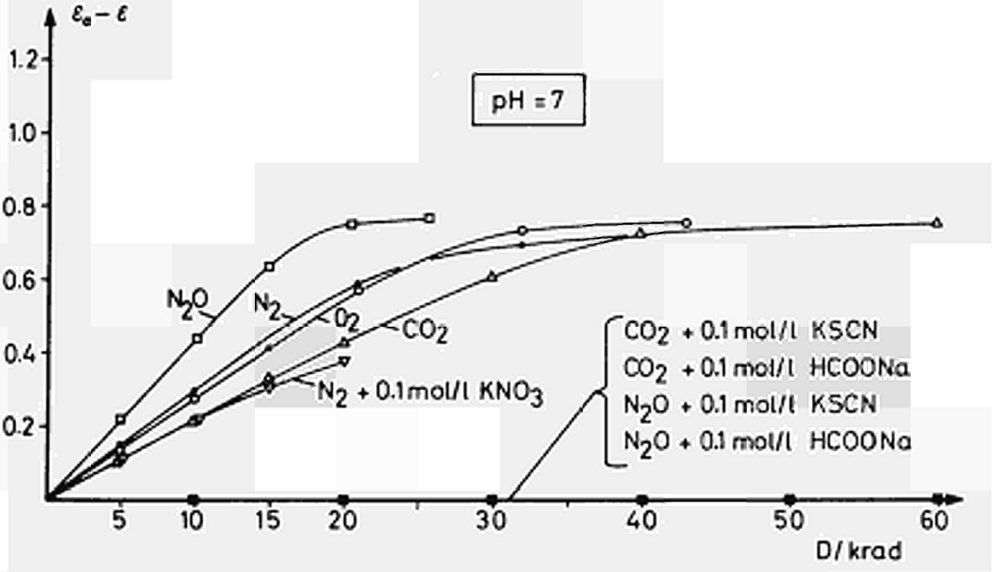

FIGURE 1 : Destruction of thymine chromophores under various conditions. $\varepsilon_o - \varepsilon$: decrease of absorbance at λ = 265 nm. D : absorbed dose.

hand, the difference $G_6(T^*)-G_1(T^*) = 1.04(100eV)^{-1}$ represents the G-value for thymine chromophores destroyed by e^-_{aq}. From these two values it can be calculated, that only 40 percent of all generated e^-_{aq} destroy the thymine chromophores. In oxygenated solutions this percentage is reduced to about 25 percent.

In CO_2-bubbled and N_2O-bubbled thymine solutions, to which 0.1 mol/l KSCN or 0.1 mol/l HCOONa is added, no thymine chromophores are destroyed. That means, that the anions SCN^- and $HCOO^-$ are effective protectors for the chromophores against OH•-radicals.

TABLE 1

The G-value $G_i(T^*)$ for the destruction of thymine chromophores under various conditions i(i=1,..,11)

i	gas	additive	pH	$G_i(T^*)/(100eV)^{-1}$
1	N_2	0.1mol/l KNO_3	7	2.66
2	N_2	-	1	2.64
3	CO_2	-	7	2.68
4	O_2	-	0	2.60
5	N_2O	-	7	5.35
6	N_2	-	7	3.70
7	O_2	-	7	3.37
8	CO_2	0.1mol/l KSCN	7	0
9	N_2O	0.1mol/l KSCN	7	0
10	CO_2	0.1mol/l HCOONa	7	0
11	N_2O	0.1mol/l HCOONa	7	0

GESELLSCHAFT FÜR STRAHLEN- UND UMWELTFORSCHUNG MBH, MÜNCHEN
Institut für Strahlenschutz, Neuherberg

Vertrag Nr.: 101 BIOC

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

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

---

Originally the contract included two smaller research projects, namely:

- the study of exoelectron emission from solid organic material
- the radiation interaction within tiny tissue volumes by computer simulations.

The first topic was already cancelled two years ago in agreement with the dosimetry group and the responsible representatives of the commission. The primary particle transport part of the second topic was taken over by contract 113 BIOC. Therefore, the group concentrated its interest in the charged particle transport and the related radiation physics and microdosimetric problems, represented by the new topic "Energy transfer in model substances and radiation effects in condensed matter".

The aim of the investigations is the calculation and measurement of local energy deposition or even the local distribution of physical and physico-chemical events as excitations, ionizations, production of radicals etc., and the analysis of their relevancy to the characterization of "radiation quality" in radiotherapy and radiobiology.

Officially 12 men-months are foreseen for the contract. About 2/3 of it were spent for the theoretical track structure analysis and the rest for theoretical and experimental work with TE-proportional counters, the conventional microdosimetric apparatus.

References:

/1/ Paretzke, H.:
Comparison of Track Structure Calculations with Experimental Results
Int. J. Rad. Eng. 4 (1974)

/2/ Paretzke, H. and G. Burger:
The Physical Basis of Cell Survival Models
2. Symp. Neutron Dosimetry Biol. Med., Neuherberg, 1974

Ergebnisse des Projekts

Leiter des Projekts und wissenschaftliche Mitarbeiter:

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

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

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The project includes three fields of investigation:

a) Theoretical ion track studies.
The programs for simulating the primary track structure of fast ions had to be adapted to a new computer of the institute. At this occasion they were improved with respect to cross sections and to their applicability to condensed media. The following problems were mainly considered:

- the search for cross sections for low energy electrons ($E < 100$ eV), the diffusion of which determines recombination
- the role of collective excitation modes of the target
- the role of phase dependent excitation states of the fast ion.

The programs, beside other applications, were used for the simulation of the transport of an electron beam inside infinite media. The theoretical results for water have been compared with experimental data in air of Grün /Z. Naturforschung 12 A, 89 (1957)/. The agreement is surprisingly good (figure 1).

Radiobiological results were critically analyzed with respect to relevant target models. A cell model is in preparation for use in computer calculations of radiation effects on the molecular level.

b) Experimental microdosimetry.
These studies have been limited to the investigation of the applicability of a TE-proportional counter for mixed field dosimetry especially at very low dose rates.

It offers an alternative approach to the twin chamber technique for the separate determination of the gamma- and neutron dose component by pulse height discrimination of electron- and proton events.

For this purpose the electron distribution in an unknwon mixed field has to be fitted by the distribution for a pure gamma source. The accuracy of the method and its limitations are still under investigation (figure 2).

c) Experimental radiation physics.
Two experiments of the laboratory dealing with the direct measurement of the lateral extension of ions tracks in gases, have been continued. In the first experiment the double differential secondary electron spectra off the axis of an ion beam traversing a gaseous target chamber

shall be measured. The apparatus is nearly complete and first measurements will be performed in 1975.

In the second experiment, the light emission pattern around an ion beam traversing a gaseous target chamber will be measured and spectroscopically analyzed. To this purpose, an existing apparatus was partly changed, improved and automatized.

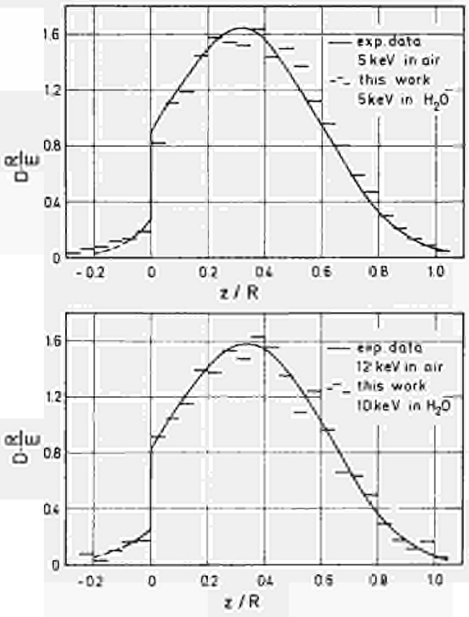

Figure 1:

Comparison between experimental and calculated scaled depth dose curves (D=depth dose, E=electron energy, R=electron range, z=distance from the origin) for a monodirectional source in an infinite medium

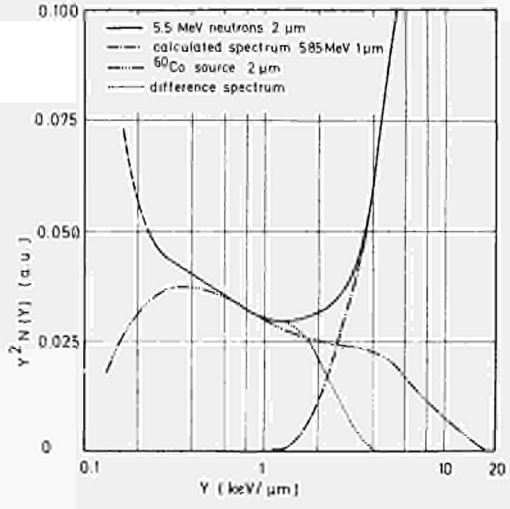

Figure 2:

Pulse height distributions in a TE-counter for accelerator neutrons with an unknwon gamma-component and for a pure Co^{60} source

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

A refined ionographic methodology has been applied to the study of secondaries distributed along α tracks recorded in nuclear emulsions. Investigations on the influence of the gelatine concentration on the distribution of the secondaries have been undertaken.

The calculated yields of secondary electrons obtained by means of an improved mixed treatment are in a good agreement with the experimental data.

The theoretical yields of recoil H-nuclei obtained by means of a LINDHARD's formula deduced from the power law interaction potential have been compared with measurements carried out in emulsions at different gelatine concentrations. The results support the hypothesis that part of the secondary events are due to proton tracks.

The mean range of 0 - 20 keV electrons in nuclear emulsion has been determined by means of a semi-empirical approach for different concentrations of the C,N,O,H compound.

PUBLICATIONS.

V.B. NDOCKO NDONGUE, E. WITTENDORP and R.V. RECHENMANN. Analytical study of ionizing secondary events along α tracks recorded in ionographic emulsions. Proc. Second Symposium on Neutron Dosimetry in Biology and Medecine, Neuherberg-München (30 sept.-4 oct.) 1974.

RESULTATS du PROJET N°1

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

The analysis of the secondary events distributed along α tracks recorded in nuclear emulsions has been improved on the basis of refined experimental and theoretical approaches. The influence of the variation in gelatine concentration of the recording medium on the experimental and theoretical data is under investigation.

Recent modifications introduced in our methodology resulted in a refinement of the observable track structures. As a consequence, an increased number of secondaries could be detected if compared with our experimental results published previously (1,2,3), the protuberances sticking out of the track-core being much more recognizable as such. Let us recall that two categories of events with radial spreads respectively of $r > \text{or} \leqslant 0.5$ μm, corresponding to the "proton" or the "electron" class have been discriminated by the introduction of criteria based on theoretical and experimental estimations concerning the range-energy relations of the secondaries, on the ejection angles, on the geometry of observation, on the track width, etc...

On figure 1 is given the histogram representing the number of δ rays per 5 μm as a function of the α particle's residual range; the agreement with the theoretical, geometrically corrected histogram is acceptable (for the considered detection threshold T_o = 5 keV). The calculated data have been obtained by an improved treatment modified if compared with the approach proposed in an earlier stage of our study (1,2,4).

Let us recall that we considered at the actual stage of our study as "free" the orbitals of the light atoms constituting the nuclear emulsion (C,N,O,H) as well as the electrons from the outer shells (M and N) of the Ag and Br atoms. The yield of the corresponding secondaries had been calculated by integrating a formula of the differential ionization cross section proposed by FANO (5).

We have applied in the case of the bound electrons (K and L shells of the Ag and Br atoms) the differential ionization cross section developed by HUUS et al. (6) which separately takes into account the ionization of each atomic shell. The number of ejected bound electrons has been determined by integrating the considered expression from the detection threshold energy to infinity.

The total δ ray yield has been obtained by summing the ejected bound and free electrons. On figure 2 is given the δ ray yield in the case of a detection threshold of T_o = 5 keV (related to the actual stage of our detection possibilities), and on figure 3 the yields also calculated by means of the proposed mixed treatment for T_o = 1, 3 and 5 keV.

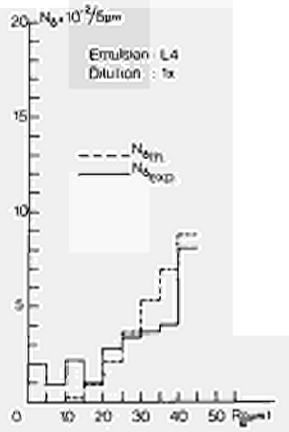

Fig. 1 : Calculated, geometrically corrected, and experimental yields of δ rays ($T_o \geq 5$ keV) per 5 µm as a function of the α particle's residual range.

Emulsion : Ilford L4 - Activated development.

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

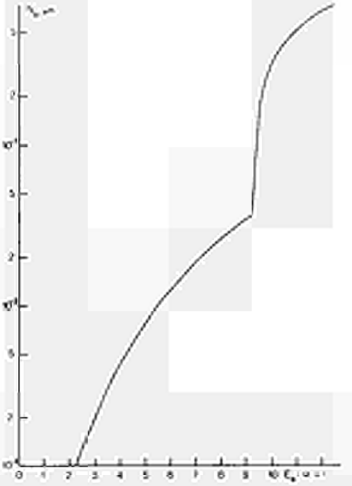

Fig. 2 : Total calculated energetic δ ray yield in standard emulsion obtained by the proposed modified mixed treatment as a function of the α particle energy E_α (threshold T_o = 5 keV).

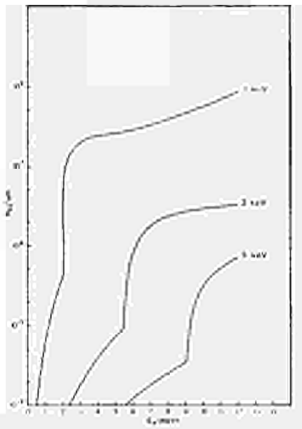

Fig. 3 : Total calculated yields of δ rays in standard emulsion for different threshold energies as a function of the α particle energy.

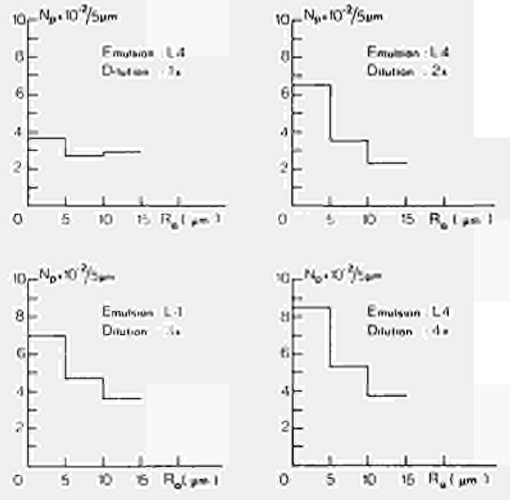

Fig. 4 : Yield (per 5 µm) of the protuberances sticking out of the primary track-core as a function of the residual range of α particles at different gelatine concentrations of L_4 emulsion : diluted 1x (51% gelatine) - diluted 2x (68% gelatine) - diluted 3x (74% gelatine) - diluted 4x (80% gelatine). These data represent only the proton tracks with radial projections on the plane of observation exceeding the criterium. The represented values are therefore always smaller than the actual total yields.

In order to support our hypothesis that at least part of the strong ionizing events may be tracks of ejected H-nuclei, we have recorded α tracks in emulsions at different gelatine concentrations.

The measurements have been carried out in the last 15 μm of the α particle's trajectory; all the protuberances sticking out of the track-core have been counted. Indeed, the probability to detect an energetic δ ray is very low, while the foreseen proton yield is at its highest in the energy region considered. The histograms representing the yields per 5 μm as a function of the residual range for different gelatine concentrations are given in figure 4.

It appears that the number of events increases with the concentration of the CNOH compound of the emulsion. The comparison of the experimental data and the yields (geometrically corrected) calculated by means of a LINDHARD's formula deduced from the power law interaction potential (7) shows an acceptable agreement if one considers that the measurements are carried out at the limits of the actual possibilities of the ionographic method.

TABLE I.

Emulsion	L_4x1	L_4x2	L_4x3	L_4x4
$r = Y_{gc}/Y_e$	1.7 ± 0.3	1.4 ± 0.2	1.2 ± 0.2	1.1 ± 0.1

Y_e : Yield of all recognizable events sticking out of the mean track diameter in the first 5 μm of the α particle's residual range.

Y_{gc} : Calculated yields, geometrically corrected, of protons with energies $E_p \geqslant 10$ keV. This energy value corresponds to a range $R_p \sim 0.15$ μm (mean half width of the track-core).

The slight decrease of r (calculated data Y_{gc}/experimental data Y_e) is probably due to the influence of following factors: a) small events are better recognizable in emulsions with higher gelatine concentrations; b) the proton range i.e. the number of protuberances sticking out of the track-core increases with the concentration in gelatine. The influence of both factors results in an increase of Y_e, i.e. a decrease of r at higher dilutions.

REFERENCES : 1) RECHENMANN R.V. Annual Report EUR.4864 (1972); RECHENMANN R.V. Annual Report EUR.5138 (1973). 2) RECHENMANN R.V., AIGUABELLA R. and WITTENDORP E. C.R. Acad. Sc. 276, Série D, 3211 (1973). 3) WITTENDORP E., HORRENBERGER A., AIGUABELLA R. and RECHENMANN R.V. Proc. 4th Symp. on Microd (Verbania-Palanza, sept. 1973), N°EUR.5122 d-e-f (1974), 189. 4) AIGUABELLA R., NDOCKO NDONGUE V. and RECHENMANN R.V. Proc. 4th Symp. on Microd. (Verbania-Palanza, sept. 1973), N°EUR.5122 d-e-f (1974), 221. 5) FANO U. Ann. Rev. Nucl. Sc. (1963), 1. 6) HUUS T., BJERREGAARD J.H. and ELBEK B. Kgl. Dans. Vid. Sel., Math. Fys. Med., Bind 30, N°17 (1956). 7) LINDHARD J., NIELSEN V., SCHARFF M. and THOMSEN P.V. Kgl. Dans. Vid. Sel., Math. Fys. Med., Bind 33, (1963), 8.

Partie B : RESULTATS du PROJET N°2

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

Methodology.

Different techniques involved in the development of a specific methodology in order to visualise the path of low-energy electrons have been developed.

Uptil now the best results have been obtained by pouring a relatively thick layer (60 µm) of nuclear emulsion on a plastic base (Falcon) and by cutting the preparation perpendicularly with the ultramicrotome after exposure and processing. Nevertheless, the results obtained are not yet reproducible enough as far as quantitative studies are concerned.

Systematic experiments are actually carried out in order to formulate a procedure resulting in the obtention of information as accurate and reliable as possible on the behaviour of electrons of intermediate energy in dense matter.

Series of experiments with ionographic emulsions at different degrees of gelatine concentration have also been undertaken.

Path lengths (R_m) of intermediate energy electrons in nuclear emulsion.

The modified semi-empirical approach proposed previously (1) has been applied to the calculation of the number of grains crossed by an electron of a given intermediate energy and to the estimation of its maximum path length (R_m) in the Ilford L4 emulsion at different gelatine concentrations ranging from L_4x1 (51 % gelatine) to L_4x8 (89 % gelatine). The results are represented on Figure 1 and Table I.

REFERENCES :

RECHENMANN R.V., NDOCKO NDONGUE V.B. and WITTENDORP E. Annual Report EUR. 5138, 1973, 31.

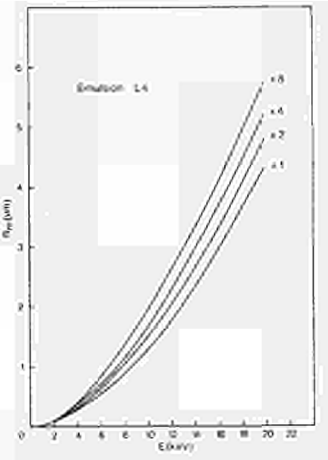

Fig. 1 : Path length as a function of energy for the intermediate energy electrons in Ilford L4 emulsion at different gelatine concentrations calculated by means of the modified semi-empirical approach.

L_4x1 : 51 % gelatine - L_4x2 : 68 % gelatine - L_4x4 : 80 % gelatine - L_4x8 : 89 % gelatine.

E(keV)	NUMBER OF CROSSED MICROCRYSTALS semi-empirical evaluations			
	L4x1	L4x2	L4x3	L4x4
1	0.3	0.3	0.3	0.3
5	2.3	2.0	1.8	1.6
10	7.1	5.0	4.0	4.0
15	14.0	10.0	7.2	7.0
20	22.6	15.7	12.0	11.0
25	33.0	22.8	17.0	16.0

Vertragspartner der Kommission:

Universität des Saarlandes
Institut für Biophysik

Nr. des Vertrages: 101 - 72 - 1 BIOC

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

Allgemeines Thema des Vertrages:

Energy transfer in biological material and model substance.

In 1974 we continued the following studies:

1. Explorations of radicals induced in various samples (water, physiological NaCl solution, culture medium and culture medium with 10 % glycerol) at a temperature of 4.2 K and their behaviour with increasing temperature.
2. Investigations of radiation-induced radicals in DNA-solutions at a temperature of 77 K and their kinetics with increasing temperature.
3. Studies of the influence of radical scavengers on radical yields and temperature dependance.
4. Investigations of radicals induced in various samples at low temperature by alpharays.

   In all of these radical studies it was tried, to determine the types of radicals and their G-values.
5. Also the studies of chromosome aberration rates induced in human lymphocytes at low temperatures were continued.

Ergebnisse des Projekts Nr. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter:

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

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

The EPR-spectra of radicals produced by X-rays (250 kV, 1,0 mms Cu) in water of 4.2 K temperature and measured at the same temperature are different from the spectra obtained in water irradiated and measured at 77 K (fig. 1). They differ in three essential points:

1. After irradiation at 4.2 K there exists a doublet. The 2 equal shaped signals of it have a large distance and are symmetrically located relatively to the central part of the spectrum, which is produced by the $OH^{\cdot}$-radicals.
2. An additional signal is observed overlaying the central part of the spectrum (fig. 1, spectrum A, left arrow (1)).
3. Another new signal has been detected, which is not existent when the measurements are performed at 77 K (fig. 1, spectrum A, right arrow (2)).

The doublet is identified by means of the characteristical EPR-data as signals of the $H^{\cdot}$-radicals.

In agreement with the results of investigations of samples which were

a) irradiated and measured at 4.2 K (spectrum A)

b) irradiated at 4.2 K, annealed to 80 K, measured at 4.2 K (spectrum B)

c) irradiated at 77 K, measured at 4.2 K (spectrum C) and

d) irradiated and measured at 77 K (spectrum D)

and in agreement with the temperature dependance of the radical concentration (fig. 2) it is supposed with a high degree

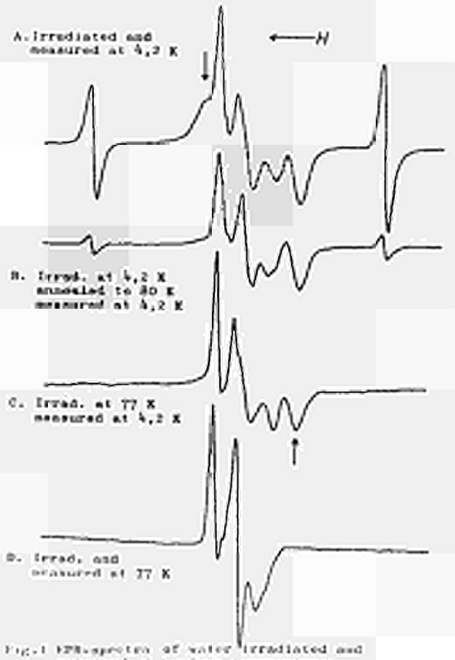

Fig.1 EPR-spectra of water irradiated and measured at various temperatures.

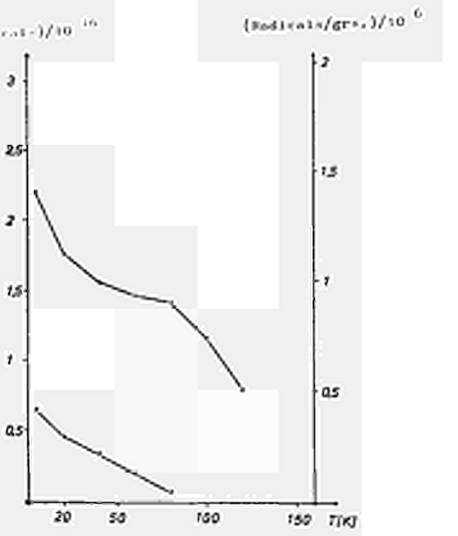

Fig.2 Temperature dependance of radicals in water

A. Central part of the spectrum
B. H- radicals
Radiation dose: 4 k rads

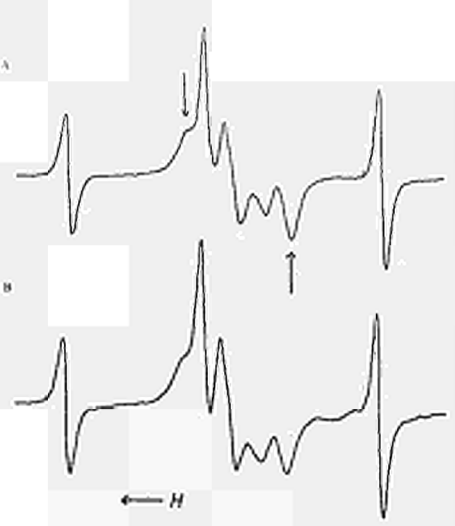

Fig.3 EPR- spectra of water irradiated at 4.2 K with A. X- rays (250 kV)
B. $^{3}_{H}$- Beta- rays
Radiation dose: 5500 rads

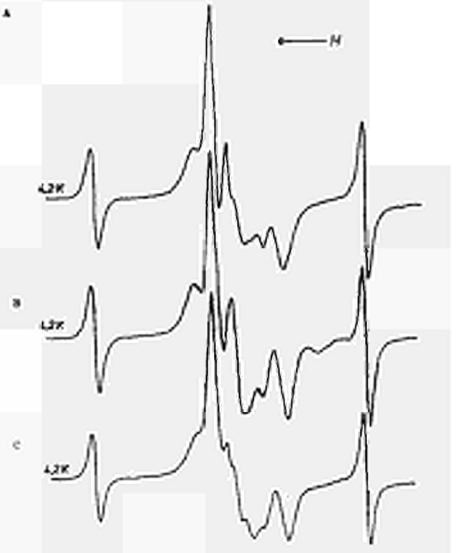

Fig. 4 EPR- spectra of various samples irradiated at 4.2 K
A. Physol, NaCl- solution
B. Culture medium
C. Culture medium with 10% glycerol.

of probability, that the additional signal marked by the left arrow (1) is due to trapped (primary) electrons. Because the other additional signal (right arrow (2)) disappears or appears in a reproducable way when the samples are cooled down to 4.2 K or warmed up to 77 K, it must be assumed that this line relates to structural changes.

By means of data of the EPR spectra, registrated immediately after irradiation at 4.2 K and after annealing to various temperatures and by the values of radical-concentrations and their temperature relationship (fig. 2) the G-values of Table 1 for e^-, H·-and OH·-radicals in irradiated water-samples are evaluated.

It is conspicuous, that the G-values of H· and OH· radicals are greater and the G-value of e^- is smaller then the corresponding values obtained in water by irradiation at room temperature. The reasons still have to be explored.

Assuming, that the radicals detected immediately after irradiation at 4.2 K are "primary" ratiolytic products produced directly by ionization or excitation,

48 % of the interactions occurring are excitations and
52 % ionizations.

EPR-investigations of water samples irradiated alternatively with X- and ^{3}H-betarays have proved, that there are no differences neither qualitatively nor quantitatively when radiation doses of equal amounts are applied (fig. 3).

Beside of water, also samples of physiological NaCl-solutions, culture medium and culture medium with 10 % glycerol were explored under the same conditions as water. The central parts of the spectra of these samples differ quantitatively and qualitatively (fig. 4) except the H·-signals.

The G-values of H·-radicals (Table 2) are equal for all of the samples whereas the G-values of the e^- and OH·-radicals are significantly different.

Beside of the EPR-measurements the exploration of chromosome-aberration rates in human lymphocytes originated under X-ray irradiation at 77 K were continued. The numerous results

obtained out of blood samples of a great variety of individuals scattered over a relative wide range. Considering this circumstances it is not easy to give a decision on what is the true dose relation ship function. But as there exists the supposition, that the radiation sensitivity of lymphocytes is different for different donors and even for the same individual when blood is withdrawn at different times, several lymphocyte cell cultures out of the same blood specimen of a donor were irradiated with increasing doses.

In all of these cases the scattering was very much reduced and now a dicision concerning the questioned dose relation ship can be given.

Concerning the connection between radicals and chromosome aberrations it is of importance to know, if the aberration rate is influenced by the period of time for which the cell cultures are stored at low temperatures (77 K).

Nine lymphocyte cultures of the same donor were irradiated at 77 K with the same dose. Three cultures were thawn immediately after irradiation, three 10 hs later and the last three cultures 20 hs after irradiation and storage at 77 K. The results in Tab. 3 don't differ within the range of statistical error.

Literatur:

FELL H. A., ESR-Tieftemperatur-Untersuchungen an wasserhaltigen biologischen Substanzen zur Bestimmung von Art, Konzentration und Kinetik der durch Bestrahlung mit Röntgen- und Betastrahlen induzierten freien Radikale.
Dissertation, Universität des Saarlandes, Saarbrücken 1974

FRIES S. ESR-Untersuchungen an bestrahlten eingefrorenen DNS-Lösungen.
(Vorl. Titel) Diplomarbeit, Universität des Saarlandes.

	$\frac{\text{Radicals}}{g \cdot rad}$	$\frac{\text{Radicals}}{100\ eV} = G$	G - values after irradiation at room temperature *
H	$1,04 \cdot 10^{12}$	1,67	0,55
OH	$2,28 \cdot 10^{12}$	3,65	2,70
e^-	$1,24 \cdot 10^{12}$	1,98	2,65

Table 1: Radical yields in water irradiated at 4,2 K

* Henglein at al.(1969)

	Water	Water + 0,9% NaCl	Culture-medium	Culture-medium + 10% glycerol
G_{e^-}	1,98	2,68	2,98	3,21
G_{OH}	3,65	4,28	4,60	4,87
G_{H}	1,67	1,60	1,63	1,66

Table 2: Radical yields of various samples irradiated at 4,2 K

Aberration rates per 100 cells	Time of storage hs		
	o,1 - 0,2	10	20
Deletions	62,1 ± 3,0	67,7 ± 3,9	55,7 ± 0,5
Dic.+tric. + rings	43,7 ± 3,4	48,2 ± 6,3	45,2 ± 8,0
Total amount of breaks	141,5 ± 2,7	158,7 ± 16,5	140,6 ± 16,7

Table 3: Meanvalues of aberration rates observed after different periods of storage time.

Laboratorio di Dosimetria e Standardizzazione

Contratto n. 068-67-6 BIOI

Capo del gruppo di ricerca: Prof. E. Casnati (*)

Tema generale del contratto:

Application of solid state devices to radiation dosimetry.

Lavori compiuti nel 1974:

The efforts have been concentrated in 1974 upon projects n. 1 and 2, regarding the application of solid state devices to dose intercomparison and to mixed fields of neutrons and gammas, respectively.

Owing to some instrumental difficulties and commercial delays, no significant progress has been achieved in project n. 3, on the use of TSEE detectors in the dosimetry interfaces.

(*) Dimissionario dal 1/11/1974

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici:

G. Scarpa, C. Giglio, P. Ientile

Titolo del progetto:

Use of solid state dosimeters as transportable instruments in dose intercomparisons.

Most of the activity carried out on this subject refers to the preparatory stage of the dose intercomparison to be undertaken in 1975 between some Italian and Romanian institutes involved in radiotherapy or radioprotection.

In this connection 16 special containers have been carefully designed and manufactured, for the simultaneous irradiation of beryllium oxide sintered discs and lithium floride powder. The geometrical and structural features of these containers are such that a fairly good homogeneity of dose can be achieved for both dosimetric materials. The plastic used is pratically light-tight, in order to avoid undue fading phenomena of BeO.

A series of experiments was also carried out in order to study the variability of TL response of BeO dosimeters. As already known for other types of solid detectors, this variability suggests that individual calibrations are to be performed.

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici:

G. Scarpa, C. Giglio, P. Ientile

Titolo del progetto:

Dosimetry of mixed fields of neutrons and gammas by solid state TL and TSEE detectors.

Risultati:

This project has been developed with a large series of experimental runs, aiming at a better knowledge of the gamma sensitivity of a number of commercial TL materials. Close attention has been put on the study of the reproducibility of response, as measured by the standard deviation of repeated readouts. For TLD 600 1x1x6 mm rads the variability coefficient was found to be between 9.5 and 15,4%, in the range from 1 up to 10,000 R. CaF_2: Mn as 1/8" square chips, displayed a lower variability coefficient ranging between 2 and 6.8%. A sistematic dose-dependance of this coefficient was observed in TLD 600 chips, with the lowest figure in the range 5-500 R (2-4%).

Another parameter taken into consideration was the individual variability of response of solid dosimeters. As far as beryllium oxide is concerned a survey of the gamma sensitivity of 200 detectors, supplied by the manufacturer as a single batch, showed a clear bimodal shape of the distribution hystogram, demonstrating that the detectors actually belonged to two different batches.

Further experiments were also carried out to better study the energy dependence of various TL dosimeters.

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

Contract No.: 128-74-1 BIOUK

Head of research team: D.H. Peirson

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

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

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

Tissue equivalent and other types of homogeneous ionisation chambers are widely used for neutron dosimetry. The aim of the present work is to measure the ionisation values in the gases commonly employed in neutron dosimetry for comparison with computed values. Measurements have been made at 15 MeV for which energy there appears to be uncertainties in the cross-sections for inelastic and non-elastic reactions in carbon and oxygen.

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

The aim of this work is to improve our knowledge of the penetration of neutrons through the body and hence to provide data on LET distributions and the dose equivalent at various points in the body. During the year measurement techniques have been developed to measure the spectrum with an organic-scintillator spectrometer using pulse shape discrimination. Theoretical calculations based upon transport and diffusion equations for a multigroup system have been started and will be compared with the experimental measurements and published calculations based upon Monte Carlo methods.

Project 3. Solid state fast neutron dosimeters

The aim of the research is to examine two new types of fast neutron dosimeter. Both use the effects of momentum transfer from fast neutrons to ions in solids, and should be capable of excellent neutron to gamma discrimination. In one system luminescence of point defects (colour centres) created directly by the neutrons is used as a measure of dose. In the second system the neutron collisions inject ions from a source compound into a suitable host solid, where their presence can be detected by the fluorescence characteristics of the injected ion in the host material.

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

The aim of the project is to obtain data on the spectra produced by a range of types of diagnostic X-ray machines such that operating conditions can be chosen to provide a suitable radiograph whilst minimising the dose to the patient. Operating conditions studied include, voltage, waveform, target angle, target type, filtration, etc and measurements of tissue and bone attenuation are being made.

Project 5. Photon spectra for dosimeter calibration

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

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

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

operating conditions of the chamber. The coordinates of the droplets are measured from stereoscopic pairs of photographs at 90^{o} and hence the position of each droplet is uniquely determined. The present year's study has been mainly devoted to overhauling the chamber and recommissioning.

Results of Project No. 1

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

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

Measurements with 15 MeV neutrons

The principal measurements for all the wall and gas compositions were made using a single parallel plate ionisation chamber with a graphite back electrode and an interchangeable front electrode. The chamber was irradiated with neutrons incident normally upon the front plate. Since recoils from the chamber wall are produced in a forward direction, the influence of the graphite back plate was very small. Hence by changing the front electrode and filling gas, it was possible to make measurements in chambers of different atomic composition. The validity of the method was checked using a thimble shaped chamber constructed wholly with walls and centre electrode of CH plastic.

An independent measurement of the neutron flux density was made using a precision uranium-238 fission chamber which was calibrated to a known neutron flux at the National Physical Laboratory. The gamma-ray contamination of the neutron beam was found to be negligible by exposing both film and thermoluminescent dosimeters.

Results

The measured values of ionisation in the different gases relative to that in acetylene agreed to better than 5% with the calculations of Dennis[1], providing confidence both in the relative values of cross sections and the W values used as input data to the calculations.

Relative values of kerma derived from the ionisation measurements are compared in Table 1 with calculation; the agreement is good.

Table 1

Comparison of measured and computed values of kerma

Ionisation chamber		Ratio $\frac{\text{Kerma in gas}}{\text{Kerma in acetylene}}$		
Gas	Wall	Experiment	Calculation	
			Dennis[1]	Bach and Caswell[2]
Acetylene	Polystyrene	1.00	1.00	1.00
Ethylene	Polythene	1.50	1.49	1.47
Ethylene/carbon dioxide	Perspex	1.03	0.99	0.98
TE Gas	TE Plastic	1.16	1.14	1.12
Carbon dioxide	Graphite	0.361	0.348	0.368

Flux densities measured with the different ionisation chambers were 8% to 13% greater than those measured with the calibrated fission chamber. This satisfactory agreement between ionisation and flux density measurement increases confidence in the methods employed for neutron dosimetry.

Publication

DELAFIELD, H.J., CHUANG, L.S., HOLT, P.D. Comparison of measured and computed values for the ionisation in various gases irradiated with 15 MeV neutrons. In: Proceedings of the second symposium on Neutron Dosimetry in Biology and Medicine, Munich, 1974. (IAEA, Vienna, 1975).

References

(1) DENNIS, J.A. Computed ionisation and kerma values in neutron irradiated gases. Phys., Med., Biol., vol.18, p.379 (1973).

(2) BACH, R.L. and CASWELL, R.S. Energy transfer to matter by neutrons. Radiat. Res., vol.35, p.1 (1968).

Results of Project No. 2

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

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

Preliminary measurements with an organic-scintillator spectrometer

In order to develop the technique a spectrometer was constructed based upon the "Owen system" of pulse shape discrimination. In this system the degree of space-charge saturation in the final dynode to anode space of the photomultiplier provides a discrimination signal. This system was tested with two sizes of liquid scintillator Type NE 213, 19 mm x 19 mm (diameter) and 51 mm x 51 mm (diameter) and a small stilbene crystal 10 mm x 10 mm (diameter). The electronic system is difficult to set up and has a lower threshold for neutrons of about 1 MeV and a dynamic range of about 20:1. A new pulse shape discriminator has therefore been developed based upon "charge comparison". This discriminator has a very high count-rate capability and is easy to set up. It should have a neutron energy threshold of at most 100 keV and a dynamic range of 100:1. A 30 cm light pipe is being tested for use with the man-phantom. Computer programs to analyse the spectrometer output have been written and tested.

Calculation of the penetration of neutron beams into tissue

We are solving the transport and diffusion equations for a multi-energy group system applied to a piece of human tissue which is irradiated by a neutron beam.

We treat the first few collisions of each neutron in the tissue as anisotropic and the full transport equation[1], which takes explicit account of the directions of the neutrons as well as of their energies, is solved algebraically for these collisions. After two scattering collisions we assume that the angular distribution of the neutrons is isotropic and we use it as a source for the numerical solution of a set of coupled diffusion equations corresponding to a set of energy intervals.

The tissue is assumed to be a rectangular block with Cartesian lattice points at unequal intervals. The diffusion equations for the lattice points yield matrix equations which are solved with the use of sparse

matrix techniques developed by A.R. Curtis and J.K. Reid[2].

The incident neutron beam is assumed to be parallel and broad compared to the mass of tissue and of constant flux, but its direction is arbitrary.

References

(1) TAIT, J.H. Neutron Transport Theory, Longmans, London 1964.

(2) CURTIS, A.R. and REID, J.K. Fortran subroutines for the solution of sparse sets of linear equations. AERE - R 6844, 1971.

Results of Project No. 3

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

Title of Project: SOLID STATE FAST NEUTRON DOSIMETERS

Direct displacement system

In this method we have chosen to explore the use of F^+ and F centres in alkaline earth oxides as luminescence dosimetry systems. These compounds are the simplest ionic crystals in which well-characterised point defects, in this case oxygen vacancies, are produced by neutron damage, without any direct production of defects by ionising radiation. A dosimeter based on these solids should be selective to neutrons, and show a response whose neutron energy dependence is close to that of tissue. Previous work at Harwell has shown that the detection of F^+ centres in MgO by photoluminescence is limited by overlapping luminescence from impurities (e.g. Fe^{3+}) in available crystals, so that fast neutron doses below 10^{12} neutrons cm^{-2} (~ 3 krad for 2 MeV neutrons) could not be detected. However, in CaO there is less interference from impurities and in the best crystals a levelling off in luminescence does not occur until 10^{10} neutrons cm^{-2}. The objective of work during 1974 has been to explore the origins of this pre-dose luminescence and to examine ways of reducing it. We have also examined the possibility of using the luminescence of F centres rather than F^+ centres, since the decay time of the luminescence is much longer (several milliseconds), which would allow time-resolved discrimination against background fluorescence from other centres. The use of thermoluminescence to detect F^+ and F centres in these oxides is also being explored.

Work on the pre-dose luminescence in CaO crystals is not yet complete and has been held up by problems with the He-Cd laser used to excite the F^+ luminescence. However, it now appears that not all the luminescence is associated with mechanical damage at surfaces, as had been first thought, and there is a bulk component which may be difficult to remove. A series of crystals from different sources remains to be examined to give a quantitative assessment of the lowest pre-dose emission which may be achieved in practice.

It has been established that one-fifth of the F^+ centres created by neutron irradiation of CaO can be converted to F centres by X-irradiation. It is therefore possible to measure dose using the F luminescence rather than the F^+ luminescence. Although a similar conversion appears to take place in MgO, we have not been able to detect any F luminescence in this compound. Equipment for thermoluminescence studies of these oxides in the temperature range 200-400°C is being assembled to investigate this form of readout process.

Ion-injection system

Work prior to 1974 concentrated on finding suitable combinations of host material and injected ion, the criteria being that the ion-host combination should be a good phosphor and at the same time the host should initially be free of impurities of the chosen ion. The latter criterion is probably the most difficult to meet, and the most successful combinations are high purity oxide hosts with rare-earth ions. The most promising system, chosen on the basis of the earlier work, is Gd^{3+} ions injected into a fused SiO_2 host. This has an advantage that, unlike single crystal hosts of MgO and CaO, Gd^{3+} fluorescence is observed after injection without requiring heat treatment. The Gd^{3+} fluorescence has a decay time of several milliseconds, so that time-resolved fluorescence can be used to discriminate against unwanted backgrounds.

To investigate a realistic dosimeter configuration, in which the interfacial area between source and host compounds is maximised, powders of Gd_2O_3 of crystallite size 10-15 nm and SiO_2 of crystallite size 5-10 nm have been prepared by electron beam evaporation and vapour phase condensation. Equal volumes of powder were then mixed together and formed into pellets by compaction. Scanning electron micrographs of the pellets suggest that individual crystallites tend to remain coagulated rather than being completely dispersed, and a further problem is that, although neither the Gd_2O_3 nor SiO_2 powders showed any detectable Gd^{3+} fluorescence before mixing, some fluorescence is observed from the pellets. There is some evidence that this may be reduced by avoiding a grinding stage in the mixing process, but it is clear that the production of well-mixed oxides without any source-host interaction will be very difficult. Some of the pellets have been irradiated with neutrons to investigate injection of ions from Gd_2O_3 into SiO_2, but measurements of the Gd^{3+} fluorescence show that any injected ions cannot be distinguished from those present in the

as-fabricated pellets before irradiation.

In view of these difficulties we are now devoting some attention to the possibility of dispersing fine powders of rare-earth oxides in a liquid monomer host, which may then be polymerized into a solid plastic pellet. We are also investigating the dispersion of a host compound (SiO_2) in a low melting point rare earth compound such as gadolinium nitrate. This work is at a very preliminary stage.

Publications

HUGHES, A.E. and PELLS, G.P. Fluorescence spectra of Gd^{3+} ions in calcium oxide. J. Phys. C. (Solid State Physics) vol.7, p.3997 (1974).

HUGHES, A.E. and PELLS, G.P. Absorption and luminescence of bismuth ions replanted into MgO and CaO. Phys. Stat. Sol.(9), vol.25, p.437 (1974).

Results of Project No. 4

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

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

Comparison of X-ray spectra and outputs from molybdenum and tungsten targets

A detailed comparison has been made of the spectra and outputs from a constant potential X-ray set with interchangeable tungsten and molybdenum targets. The spectra after filtration by various thicknesses of molybdenum and aluminium have been computed from measured unfiltered spectra using the appropriate attenuation coefficients generated by a computer program. Previous studies have demonstrated that this is a valid procedure. Thus conditions most suitable for mammography and the radiography of specimens can be determined.

Spectrum variations during the voltage waveform cycle

Spectral measurements are being extended to those for equipments operating from a pulsating rather than a constant voltage. In these cases the spectrum and exposure rate may vary considerable over the voltage waveform cycle. Conventional spectrometry will produce a mean spectrum which is only a true mean when dead time corrections do not vary significantly between the maximum and minimum of the exposure rate. Prototype circuits have been developed such that the instantaneous spectrum can be measured for selected points in the waveform cycle. Thus the true mean spectrum can be synthesised and the peak voltage and the ripple measured accurately.

Measurement of tissue and bone attenuation for X-rays up to 100 keV

The production of suitable phantoms simulating the human body is required for many aspects of dosimetry and radiography together with data on their absorption characteristics. Using beams of fluorescent X-radiation at 9.8 and 17.4 keV suitably collimated and detected by the spectrometry system, narrow beam attenuation coefficients have been measured for 45 materials. These included existing and new tissue substitutes together

with other materials such as perspex, water and polythene commonly used in dosimetry. This investigation has been extended to various human tissues including fat and bone. Techniques have been developed for preparing and mounting these specimens and a series of measurements completed for 13 tissues. The results for bone measured for a range of energies between 9.9 keV and 67.2 keV agree closely with values calculated for Woodward's formulation[1] but differ significantly from those given in ICRU[2]. Analysis of the other results is proceeding.

Calibration of a penetrameter used for checking X-ray beam quality

A penetrameter, (step wedge-film device)[3], is becoming widely used in hospitals to check the correct setting of X-ray tube voltage. The device is calibrated initially against a constant potential generator and the National Physical Laboratory offer this service for such potentials between 50 kV and 120 kV. There is considerable interest in the effect of pulsating waveforms on the penetrameter assessment of voltage and in using the device for higher voltages. The spectrometer has been used to measure a range of peak voltages from a (Watson Type MX2) self rectified set without high voltage cables, a similar Picker unit with cables the capacity of which provides some smoothing, and a Pantak constant voltage unit up to 400 kV. These results are being compared with an assessment of the voltages carried out with a penetrameter for the same operating conditions. Indications are that for the Watson equipment a penetrameter calibrated with constant potential underestimates the peak voltage by a few kilovolts over the range 50 kV to 80 kV and for currents between 1 mA and 15 mA.

Publication

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

References

(1) WOODWARD, H.Q. The elementary composition of human cortical bone. Health Physics, vol.8, p.513 (1962).

(2) Radiation Dosimetry: X-rays generated at potentials of 5-150 kV. ICRU Report 17, p.26 (1970) Washington.

(3) ARDRAN, G.M. and CROOKS, H.E. Checking diagnostic X-ray beam quality. Br. J. Radiol. vol.41, p.193 (1968).

Results of Project No. 5

Head of Project and scientific staff: L.H.J. Peaple
T.J. Crosby
R. Birch

Title of Project: PHOTON SPECTRA FOR DOSEMETER CALIBRATION

Filtered X-radiations

A new series of filtered radiations producing low exposure rates for the calibration of protection level dosemeters has been designed and proposed to the International Standards Organisation. Tube voltages and filtrations have been selected for 8 radiations covering the range 30 keV to 210 keV, each radiation having a width equal to 20% of its mean value and producing an exposure rate at 1 metre of approximately 40 mR h^{-1} mA^{-1}.

In order to obtain reproducible spectra and exposure rates the tube voltage under load must be measured and set accurately. Accepted methods of measurement include the use of a calibrated resistor chain and voltmeter and the determination of the maximum photon energy by spectrometry. These two methods have been compared for an installation employing a suitable constant potential generator. At low tube currents there is no voltage ripple and the two methods are in agreement. Discrepancies occur as the current, and hence the ripple, increases since the meter indicates an average and the spectrometer the peak value. The effect of ripple on the mean spectrum and exposure rate and the choice of mean or peak voltage has been investigated for the low exposure rate series.

When ripple is present the measured spectrum and exposure rate may differ significantly from that applicable to the extremes of voltage. Circuits have been designed for measurement of the instantaneous spectrum and hence exposure rate at selected points in the waveform cycle. A prototype equipment has been built and preliminary tests indicate that it performs satisfactorily.

Fluorescent X-radiations

The International Standard Organisation's fluorescent series contains 10 radiations, each characterised by the material of its radiator and selective filter, their thicknesses and the X-ray tube voltage. These

parameters affect the radiation purity and spectrum measurements have been carried out to investigate anomalies between the quoted values and those which have been obtained in practice. In particular, work has been concentrated on the selective filter, the thickness of which determines the ratio of the K_β to K_α lines as well as affecting the overall purity. A computer program has been written to calculate this ratio and for filters readily available in the form of metallic foils, good agreement has been obtained between calculated and measured values. Discrepancies still exist for filters of strontium, tellurium, cerium, gadolinium and ytterbium formed from powders dispersed in a plastic binder, due to inaccuracies and inhomogeneities introduced in the manufacturing process. Various methods and techniques for improving the quality of these filters have been investigated, involving different types of resin bonding and including hot pressings. Recently a method developed at St. Bartholomew's Hospital for the manufacture of tissue substitutes has been used and the results are very promising. The full range of radiators and filters are being produced by this process.

Results of Project No. 6

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

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

The present study was begun in May 1974. As the chamber had been out of use for some time it has been overhauled and recommissioned. Possible ways of improving the quality of the data produced have been examined with particular reference to the following points: (a) The gas mixture previously used was not tissue equivalent and therefore interpretation of results in terms of tissue-like materials was difficult, (b) The original gas composition could not be determined very accurately after it had reached equilibrium in the chamber due to the unknown absorption of the water and ethanol on the walls of the sampling and measurement devices, (c) The precision required on the measurement of the positions of the droplet images in order to obtain three dimensional coordinates from stereoscopic (90^{o}) pairs of photographs was close to the limitations ($\pm$ 1 μm) of the best measuring machine available (Vanguard, Rutherford Lab).

A new gas mixture has been devised which is not only tissue equivalent but also reduces the problems of measurement of gas composition. This mixture consists of (approximate pressures in mm of Hg) 19.4 mm H_2, 11.3 mm O_2, 10.1 mm C_2H_5OH and 0.8 mm N_2. The working composition can be determined from the initial composition and any pressure drop, since the only component likely to be significantly absorbed in the oil of the chamber is ethanol. External sampling and analysis of the mixture will also be easier since no water is present.

The electron density of the new mixture is 97% of that previously used so that the droplet separation will be similar. However it should be possible to reduce the overall density of this mixture giving an effective increase in magnification of the photographs. Studies of the optical system (flask, camera and film) have shown that a further increase in linear magnification by 1.4 could be achieved if required.

Preliminary photographs of droplets produced by X-ray from an iron-55 source have been produced. Present work is concentrated on the production

of aluminium X-rays (since the commercial devices previously used can no longer be obtained) and on optimising chamber conditions and optical and electronic parameters in order to obtain the best photographs.

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

A detailed examination of the effect of annealing conditions on the thermoluminescence properties of lithium fluoride and lithium borate phosphors has been carried out in project 1. Lithium fluoride has been cooled at different rates within the range 0.1°C/minute to $2.5 \times 10^{4^{\circ}}$C/minute under controlled conditions. The effect of environmental gases and vapours on lithium fluoride and lithium borate:manganese stored at temperatures up to 80°C has also been investigated. A technique has been developed which allows the original radiation dose received by TLD lithium fluoride dosemeters to be re-estimated within the millirad range.

In Project 2, the calculation of local bronchial deposition of radon daughter aerosols was examined by direct measurement of bronchial deposition in excised lungs. The results indicate that although the initial deposition can be calculated, ionic material can subsequently diffuse freely through the mucosa. This factor must also be quantified in order to define the dose delivered to the bronchial epithelium in miners exposed to radon daughters. This is of interest (1) for comparison with the epidemiology of lung cancer, and (2) in relation to the possible carcinogenic effects of other α-emitters in lung.

Results of Project No.: 1

Head of Project and Scientific Staff: E. W. Mason

A. F. McKinlay

Title of Project: Solid State Physics Processes Underlying the Properties of Some Materials used in Thermoluminescence Dosimetry

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Cooling Rate Effects in TLD grade Lithium Fluoride(LiF)

It has been shown that the transfer of thermoluminescence signal from shallow to deeper traps in LiF during storage is a function of the rate at which the phosphor is cooled after a high temperature anneal. Figure 1 compares the heights of the resolved glow peaks conventionally numbered 2 and 5, (a) immediately after cooling and (b) after further storage for one hour at 100°C. For cooling rates of less than 1000°C/minute there is no substantial growth of peak 5 at the expense of peak 2. Only at the very fastest cooling rate is there any approach to the figure of about 30% increase in peak 5 which has been reported. From a practical dosimetry view-point it is clear that a very rapid cooling could result in sensitivity changes during the issue period of a LiF dosemeter and should be avoided.

The rapid decline in peak 5 at slower cooling rates may be due partially to the effect of aggregation processes on both traps and luminescence centres but it is likely that competition between traps for electrons is an important factor which probably also contributes to the decline of peak 5 above 1000°C/minute.

Effect of Enviornmental Gases and Vapours on TLD Grade Lithium Fluoride (LiF) + Lithium Borate:Manganese ($Li_2B_4O_7$:Mn)

Lithium fluoride and lithium borate phosphors have been stored under a variety of controlled temperature and humidity conditions. The thermoluminescence sensitivity of lithium borate powder is unstable in humid conditions even at room temperature. In contrast lithium fluoride powder is not significantly affected by humidity for temperatures up to 40°C but at 80°C the thermoluminescence sensitivity is markedly affected in comparison with powder stored under dry conditions.

Related experiments have shown that the loss of sensitivity during storage of LiF powder at 80°C is even greater in vacuum, nitrogen or argon than in wet or dry air. In a dry oxygen atmosphere results comparable to those for wet air have been obtained indicating the importance of both O^{2-} and OH^- ions or molecules in determining thermoluminescence sensitivity in LiF.

No significant changes in the glow curve have been observed during the experiment suggesting that either OH^- ions or O^{2-} ions play an important role in the emission process in LiF. A hypothesis currently being examined assumes that the emission centres consist of Ti^{3+} ions associated with up to three impurity ions (probably OH^- or O^{2-}). These $Ti(OH)_n$ or $Ti(O^{2-})_n$ complexes will normally form positively charged or neutral units.

However positively charged centres capable of attracting those electrons freed during read-out are created as the temperature is increased. At low temperatures some complexes with a single nett positive charge are present. As the temperature is increased more OH^- (or O^{2-}) ions dissociate from the Ti^{3+} ions giving rise to greater concentrations of doubly and then triply charged complexes. The observation of other workers that the emission spectrum is different for the different glow peaks would support this model. The observations reported in the literature that at 500°C or 600°C the presence of OH^- ions in the atmosphere reduces the thermoluminescence sensitivity of LiF may be a consequence of concentration quenching.

Re-estimation of Dose in LiF Dosemeters

The optical bleaching technique used for the re-estimation of the radiation dose received by LiF thermoluminescence dosemeters has been extended to the extruded ribbon and Teflon disc form of dosemeter. The threshold of detection for re-estimation is 1.10 rads for Teflon discs and 2.80 rads for extruded ribbon dosemeters when the uv bleaching exposure is carried out at ambient temperature.

The sensitivity of the technique is greatly enhanced by performing the bleaching at higher temperatures (figure 2). For bleaching at 80°C a threshold for re-estimation of a few hundred millirads has been measured and a 750 mrad dose has been re-estimated for 10 LiF:Teflon discs with a standard deviation for the group of 13%.

Publications:

Correct handling of thermoluminescence LiF:Teflon Dosemeters. E. W. Mason, T. O. Marshall, K. B. Shaw, T. E. Blackman, T. F. Johns and H. E. Preston. Brit. J. Radiology, 47, 361. 1974.

The effect of temperature and humidity on lithium fluoride and lithium borate thermoluminescence dosemeters. E. W. Mason, A. F. McKinlay, I. Clark and D. Saunders. Proc. 4th Int. Conf. on Luminescence Dosimetry, Krakow, Poland. 1974.

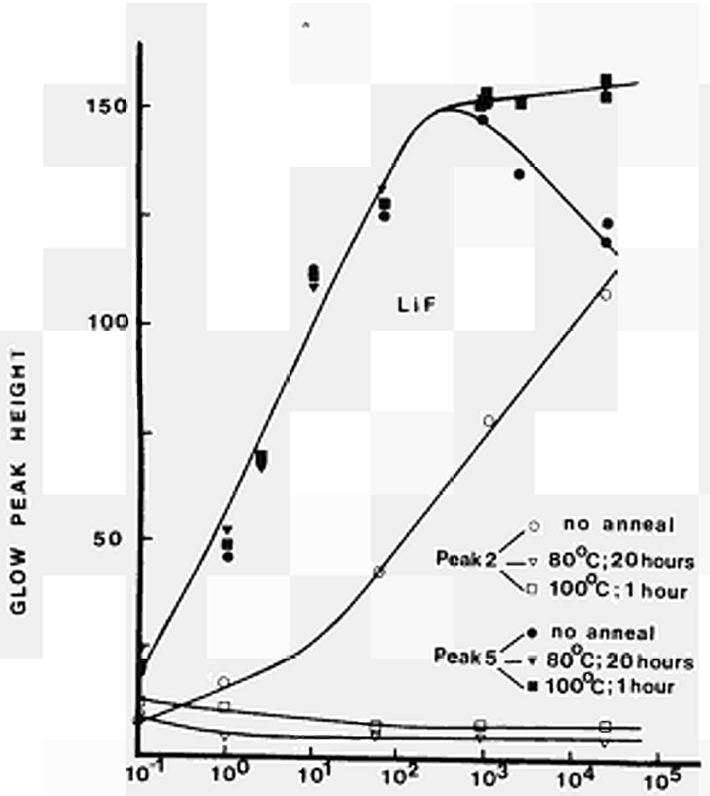

Figure 1

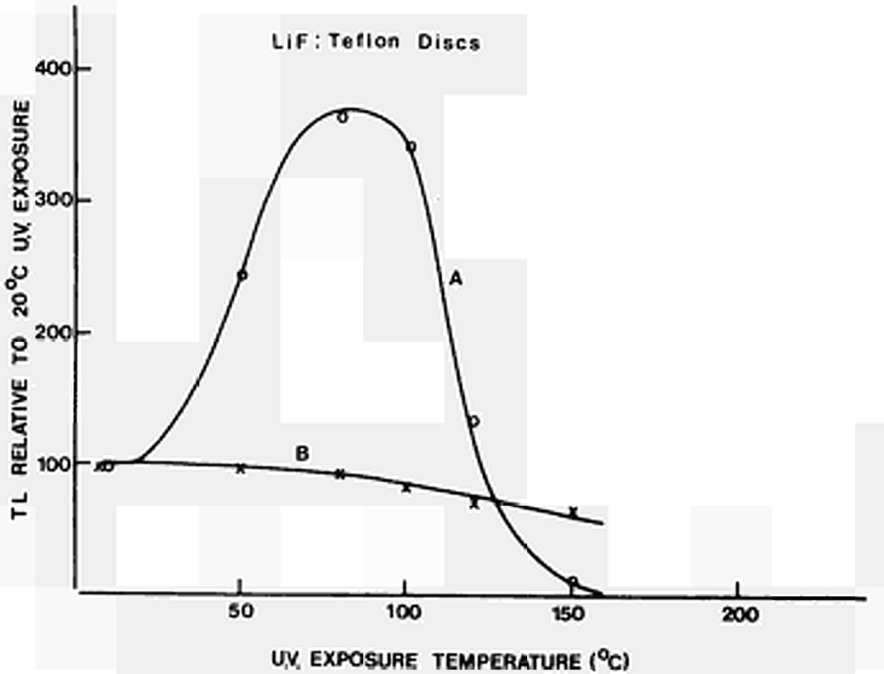

Figure 2. A. Thermoluminescence induced by uv light bleaching of LiF dosemeters as a function of uv exposure temperature. Dosemeters previously read.
B. Thermoluminescence of LiF dosemeters after read-out and storage at various temperatures in darkness.

Results of Project No.: 2

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

Title of Project: The deposition of radioactive aerosols in lungs

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Apparatus for controlled ventilation of excised lungs and exposure to natural aerosols

Construction of an artificial thorax and an automatic negative pressure ventilator for excised pig lungs was completed. A system to collect exhaled aerosols for measurement with low particle loss and low impedance to breathing has been designed and constructed. An aerosol generator delivering either free ions of the thoron daughter, Pb-212, or condensation nuclei from room air tagged with Pb-212 (AMD in the range 0.08 μm-0.25 μm) has been constructed. These aerosols simulate the radon daughter aerosols to which miners are exposed.

Bronchial deposition of free ions and condensation nuclei

Local bronchial deposition of Pb-212 was measured in freshly obtained, excised lungs, ventilated with aerosols, at constant tidal volume and respiratory frequency. After exposure, approximately 100 individual bronchi were dissected from each lung and their dimensions and Pb-212 γ-activity measured. For each bronchus, the relative ventilation was estimated from the fraction of inhaled sub-micron particles deposited distally in the fine terminal airways. Thus, the probability of particle deposition per cm² of bronchial epithelium was measured and the average air flow-rate in bronchi estimated over a wide range of bronchial size and flow-rates. Measured particle deposition for each bronchus was compared with that calculated assuming diffusional deposition under laminar flow conditions. Figures 1 and 2 summarise results for condensation nuclei.

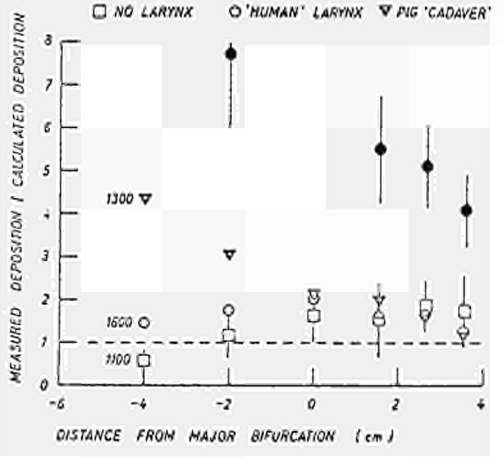

FIGURE 1

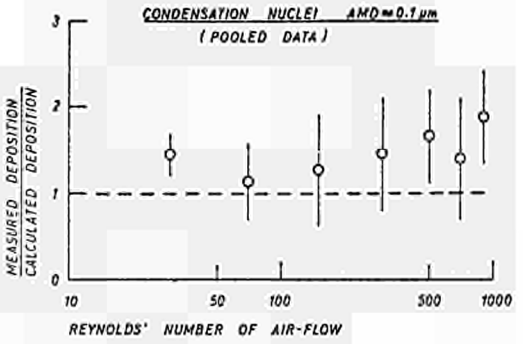

FIGURE 2

In fig. 1 the ratio of measured:calculated deposition is plotted for 3 sections of trachea and the first 3 bronchial generations, under different entry conditions. The solid points are the markedly higher deposition values reported by Jacobi (1972) in hollow rubber casts without a larynx. For excised lungs, deposition is within a factor 2 of calculated values with the exception of the first 4 cm of trachea when preceded by a 'cadaveric' larynx. This represents a more severe airway constriction than that encountered 'in vivo'. In fig. 2 the ratio of measured:calculated deposition is plotted as a function of Reynolds' number for bronchi sampled as far as the 10th generation. For the majority of bronchi sampled ($Re < 300$) the ratio of measured:calculated deposition is less than 1.3:1. The total deposition of condensation nuclei measured in excised pig lung increased monotonically from 15% at 150 ml tidal volume to 40% at 1500 ml, compared with 16% and 48% respectively, calculated for human lung with Weibel Model 'A' dimensions.

We conclude from this work that the local bronchial deposition of condensation nuclei can be calculated with adequate precision for the initial stage in the calculation of dose to the bronchial epithelium.

Similar results were obtained for free ions deposited in the trachea. However, in small bronchi (~2 mm dia.) the average observed Pb-212 concentration decreased to only 12% of the calculated value and, furthermore, Pb-212 was uniformly distributed through the mucosa and bronchial wall. Thus, in vitro, Pb-212 ions diffused radially from their deposition site on the epithelial surface, through the mucosa and bronchial wall, into the lung parenchyma, where their initial concentration was an order of magnitude lower. Equilibrium concentrations were attained in about 2 hours across a bronchial wall thickness of 0.5 mm. In vivo, the diffusion distance to blood capillaries in the mucosa is less than 0.2 mm. Thus, diffusion of ionic material, deposited in the T-B region, into blood may well have a significant effect on clearance and also on the distribution of α-activity relative to the sensitive basal cells.

Publications:

A radon daughter monitor for use in mines. A.C. James and J. C. Strong. USAEC CONF-730907, pp.932-938. 1974.

Bronchial deposition of free ions and sub-micron particles studied in excised lung. A.C. James. Abstract submitted for BOHS 4th International Symposium. 1975.

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

Contract No. : 135-74-7 BIOUK

Head of research team : Dr. B.M. Wheatley.

General subject of Contract : Production of intermediate energy neutrons.

A novel source of intermediate energy neutrons has been produced. The neutrons originate from an antimony-beryllium source located at the centre of spherical assembly of water and boron. Computer programmes were used firstly to define an appropriate combination of moderating and absorbing materials and subsequently to predict the number and spectrum of neutrons emitted from a fabricated device.

The device consists of a water filled boron loaded plastic shell with an antimony-beryllium source located at the centre.

The source, which will be used for instrument calibration, could also be used for microdosimetric studies, although the high gamma to neutron dose ratio and the low neutron yield make it inappropriate for radiobiological experimentation.

Concurrently studies have been made of many other intermediate energy neutron sources with emphasis on those which might be suitable for radiobiological experimentation. Progress in related studies at other laboratories is being actively monitored.

Results of Project No. :	135-74-7 BIOUK
Head of Project and scientific staff :	J.R. Harvey, R.C. Bending. (total effort 1.2 men starting 1st July, 1974).
Title of Project :	Investigation of sources of intermediate energy neutrons.

The first stage in this project has been the production of a source of neutrons in the intermediate energy range. It was initially decided that the design would be based on an antimony-beryllium source inside a moderating assembly with an outer shell consisting of a low energy neutron absorber. Calculations were therefore made with three independent Monte Carlo computer programmes for notional assemblies consisting of an antimony-beryllium source inside spherical light and heavy water moderators of a range of sizes surrounded by various spherical shells of boron 10.

It was concluded that a suitable device would consist of a 4 cm radius light water sphere surrounded by a 1 mm thick shell of boron 10. An appropriate shell was constructed from curved pentagonal components moulded from a mixture of bakelite, a proprietary plastic, and boron carbide. These components were cemented together to form a shell which could be filled with water and handled with remote handling equipment.

Further Monte Carlo calculations based on the measured atomic composition of the shell were used to predict the number and spectrum of neutrons emitted from the assembly. 18% of the antimony-beryllium source neutrons escape from the assembly, and the spectrum, which extends over a number of decades of energy, has an effective energy of 0.5 keV. A report describing this work is in early draft form.

The review of other methods of producing intermediate energy neutrons has lead to the conclusion that a high flux reactor could, in principle be used to provide a range of sources of roughly monoenergetic neutrons. An appropriate method of monochromating the spectrum is with various combinations of scattering and absorbing elements. A manganese scatterer in conjunction with a scandium filter for example can be used to produce neutrons at around 2 keV.

Contractant de la Commission :

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

N° du contrat : 065-72-01-PSTC

Chef du groupe de recherche : G. SOUDAIN

Thème général du contrat :

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

Le programme proposé pour 1974 prévoyait la détermination de la réponse de l'alumine radiothermoluminescente :

- aux neutrons thermiques,
- aux neutrons rapides monocinétiques d'énergies variées (expérimentations effectuées auprès du Van de Graaf du CEN-Cadarache),

et l'utilisation de l'alumine dans un cas réel de champ mixte auprès de l'installation SILENE à Valduc.

En raison de difficultés d'accès au Van de Graaf de Cadarache, nous avons fait des irradiations avec des sources de ^{252}Cf, de Pu-Be et un accélérateur donnant des neutrons de 14 MeV. De plus nous avons eu la possibilité d'étudier d'autres détecteurs couramment employés pour lesquels nous donnons également des résultats.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques :

G.PORTAL, R.MEDIONI

Titre du projet :

Dosimétrie des photons en présence des neutrons

Les paramètres qui nous sont apparus comme déterminants pour cette étude sont la sensibilité intrinsèque du détecteur et l'influence du conteneurdans lequel il est irradié. L'expérience montre en effet que la nature et les dimensions du conteneur utilisé jouent un rôle important. En particulier, les protons générés par les neutrons dans les matériaux hydrogénés contribuent à une part importante de la dose enregistrée par le dosimètre lorsque le conteneur est de faible diamètre. La réponse la plus faible dans un champ de neutrons donné a été obtenue pour les conteneurs non hydrogénés.

En 1974 notre étude a porté sur les sources de neutrons suivantes :

- neutrons thermiques du réacteur piscine Triton (CEN-FAR)
- sources de Californium 252 et de Pu-Be,
- neutrons de 14,7 MeV.

Parallèlement à l'alumine, nous avons également étudié la sensibilité aux neutrons d'autres produits thermoluminescents (SO_4Ca, Dy; $^{naturel}LiF$, ^{7}LiF), de détecteurs photoluminescents (PB33) et photographiques (Kodak-Pathé, type 1).

Le bruit de fond dû au champ gamma des sources utilisées a été déterminé à partir d'un compteur GM construit d'après les spécifications de WAGNER et HURST. Pour les neutrons thermiques, leur contribution est donnée par la différence des lectures de dosimètres identiques placés sous écrans de graphite et de lithium.

Le tableau ci-dessous résume les résultats obtenus. Les sensibilités intrinsèques sont exprimées en équivalents röntgen de ^{60}Co pour 1 rad de neutron (kerma).

A partir de ces premiers résultats, il nous a été possible de déterminer le champ γ autour du réacteur Silène de Valduc en décomposant le spectre de neutrons en une partie thermique et une partie rapide assimilable à un spectre de fission.

Pour les mesures dans le rayonnement émis par ce réacteur, nous proposons l'alumine comme dosimètre de routine car elle est parfaitement adaptée aux gammes des doses de photons à mesurer et sa sensibilité aux neutrons est faible.

	Al_2O_3	$CaSO_4$	^7LiF	^{nat}LiF	PB 33	KODAK-PATHE émulsion "K"	KODAK-PATHE émulsion "D"
Thermiques	1,8	2,2	9,2	1650	33		
^{252}Cf	0,014	0,011	0,027	0,060	0,01	0,13	0,074
Pu Be	0,011	0,034	0,059	0,078	0,005	0,13	0,11
14,7 MeV	0,18	0,10	0,11	0,14	0,049		0,072

PUBLICATIONS

F. SPURNY, G.PORTAL, Sensibilité de divers matériaux RTL et RPL aux neutrons thermiques, 4ème Conférence Internationale sur la Dosimétrie par Thermoluminescence, 27-3I août 1974, Cracovie (Pologne).

F. SPURNY, R. MEDIONI, G.PORTAL, Les sensibilités des divers détecteurs thermoluminescents, photoluminescents et photographiques aux neutrons, 2ème Symposium sur la Dosimétrie des neutrons en Biologie et en Médecine, 30 septembre au 4 octobre 1974, Neuherberg-Munich (R.F.A.)

COMITATO NAZIONALE PER L'ENERGIA NUCLEARE

LABORATORIO FISICA SANITARIA, BOLOGNA (Italy)

CONTRACT No.: 065-72-1 PSTC

Leader of the Research Projects: G. Busuoli

General Subject of the Contract:

STUDIES ON NEW DETECTORS USEFUL FOR PERSONAL DOSIMETRY

Project No.1

Project Leader: G.Busuoli

Project Title: Applicability of TSEE Detectors to Personal Dosimetry

Experimental Results during 1974

The experiments performed during 1973 showed that, by coating the BeO discs with a thin layer of graphite, the reproducibility of the measurements had been greatly improved. In fact the standard deviations were lowered from 40%, for alpha particle doses of about 1 rad, to few percent for the same doses of Co-60 gamma-rays.

The graphite layer however, presents the disadvantage of decreasing the sensitivity of BeO discs. Thus we have modified the counter as suggested by Gammage et al (°) by putting a conductive layer on the upper part of the heater. A negative potential is then applied to this electrode promoting the escape of the detrapped electrons and increasing the detection efficiency.

This device would also improve the reproducibility both for runs on successive the same detector and for runs on different detectors.

We have then determined the EE emission as a function of the negative voltage applied to the electrode. In fig. 1 are shown the experimental results. The emission increases asymptotically;we established the working conditions at 90 volts.In order to minimize the noise introduced in the counter, this negative voltage was supplied by dry batteries. Under these conditions we have determined again the counter plateau which

(°) R.B.Gammage, J.S.Cheka "A Practical TSEE Dosimetry System Based on BeO" Fourth Int. Symp. on Exoelectron Emission and Dosimetry Liblice, Prague 1973.

has not shown appreciable variations, as expected. The increase in sensitivity with the voltage applied has been estimated of the order of a factor of 3.

Afterwards we have made reproducibility tests using carbon coated discs and after exposures to several undred mR of Co-60 gamma-rays. Appreciable improvements in comparison with the measurements without the applied voltage have not been found, with reduction of the standard deviations to the order of 5-7%. We have found that with the voltage applied,the values between the detectors are more uniform. In addition the emission of spurious flashes(not due to the irradiation) of electrons at high intensities is not as frequent as before.

At present we are performing tests on BeO uncoated discs to control if the negative electrode is beneficial also in this case. The results are still incomplete and it is not possible as yet to draw a definite conclusion on this subject.

As concerns the TL measurements on BeO discs, we are modifying the reader in order to increase its sensitivity at the wavelenght of the light emitted by the phosphor (the maximum is at approximately 260 nm). This increased sensitivity will put the BeO discs in a favourable position for the design of a two element personal dosimeter (non discriminating basic dosimeter) for long period monitoring (e.g. 3 months).

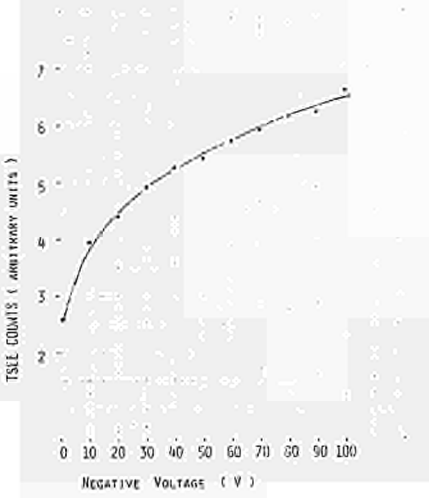

FIG. 1

Prject No.2
Project Leader: A.Cavallini
Project Title: Criticality Accident Dosimetry by Plastic Track Detectors

Experimental results during 1974

Last year we have tested the characteristics of plastic detectors useful for neutron dosimetry taking into accont two types of plastics: cellulose nitrate and triacetate. After establishing the best etching and reading conditions for alfa particles of different energies,during 1974 we have determined the sensitivity of these detectors both to thermal and fast neutrons. At present we have studied only the cellulose nitrate but cellulose triacetate will be in the next future similarly investigated. We have prepared (n, alfa) converters by coating 0.2 mm thick alluminum foils with a LiF layer of few tenths of milligram per sq. centimeter surface density;alluminum does not perturb the neutron flux.

In order to measure the efficiency of these converters to thermal neutrons, a nitrate foil was placed at close contact with the fluoride layer. Those detectors were then exposed inside a polyethylene cube in which are placed 3 Am-Be sources of 10^7 n/sec each. The thermal flux in a cavity in the cube is flat and of 2.6×10^4 cm^{-2} sec^{-1}. The readings of the etched detectors have shown that the efficiency is very high, equal to 320.000 tracks cm^{-2} rem^{-1}. This means that it is possible to use this detector for general purpose dosimetry, and just for accident dosimetry.

This same detector-converter pair has been studied to investigate the response to fast neutrons by placing the dosimeters on a phantom. The actual dosimeter consists in sequence proceding toward the phantom of an alluminum foil coated with LiF, a cellulose nitrate foil, a Cd filter, a second LiF coated Al foil and finally a second cellulose nitrate foil.This allows a good discrimination be achieved between the neutrons reflected by the phantom (second plastic detector) and the neutrons incoming directly from the source (first plastic detector).

A set of irradiations have been performed with a Pu-Li source (mean energy$\sim$ 0.2 MeV), Cf-252 (fission spectrum) and Am-Be (about 4.5 MeV). The experimental measurements are summarized in the table.

	Thermal Neutrons	Pu-Li		Cf		Am-Be	
		direct	reflected	direct	reflected	direct	reflected
Track/cm^2 rem	320.000	780	6840	320	1400	400	870

As shown in the table the energy dependence is strong. However it seems possible to gain information on the neutron energy from the ratio between the tracks density due to direct neutrons and the track density due to reflected neutrons. As indicated in the 1975 program other converters will be investigated but it seems already feasible a neutron dosimeter on a large energy range, and a dose ranging from about 200 mrem up to several hundred rems. In fact irradiations with alpha particles have shown that large dose values can be evaluated by densitometric measurements and that a considerable interval of overlapping exists between track counting and optical density measirements.

Contractant de la Commission : C.E.N. / Cadarache
(CEA - France)

N° du contrat : 65-72-1 PSTC

Chef du Groupe de Recherche : Mr Michel BRICKA

Description générale succinte des travaux réalisés :

Ainsi que prévu, la détermination des courbes de réponse a été poursuivie pour les matériels à compteurs hélium 3 mis au point les années précédentes.

Les résultats, pour les neutrons thermiques et pour la bande d'énergies 10 keV - 600 keV , confirment les calculs de R. CAIZERGUES et de J. LAMBERIEUX et peuvent être considérés comme définitifs.

Pour les énergies supérieures à 600 keV, les mesures s'écartent assez notablement des valeurs calculées, ainsi que l'avaient montré les résultats obtenus antérieurement. Quelques campagnes de mesures supplémentaires seront donc nécessaires avant de pouvoir proposer des formes de courbes définitives.

L'étude de la bande des énergies épicadmiques (0,4 eV - 10 keV) a été entreprise mais n'a pas donné, à ce jour, de résultats très satisfaisants. On obtient facilement des valeurs relatives, mais la mesure précise des fluences dans cette bande d'énergies pose quelques problèmes.

On a procédé à l'étalonnage des monosphères de 10" et 4,2", utilisés actuellement, faute de matériels plus performants, pour la mesure des fluences et doses. La dynamique de ces sphères est suffisante pour permettre d'intéressantes mesures sur les sources pulsées.

Les programmes de calcul SESR 1590 et SNAC 003 ont été ajustés, permettant d'établir une première série de formules linéaires d'exploitation. Ces formules ne sont qu'approchées, la forme définitive des courbes de réponse n'étant pas encore établie pour les matériels à compteurs hélium 3.

Un projet de compteur rem a été lancé et une première série de mesures a été faite, concernant l'effet de perforations sur la courbe de réponse d'une sphère de 10". Ce projet a été discuté avec le Groupe de Recherche de Jülich (KFA), qui serait susceptible de traiter l'aspect calcul de ce problème.

Liste des publications 1974 :

(1) - M. MOURGUES - La mesure des neutrons par compteurs à hélium 3 sous modérateurs sphériques.
Second Symposium on Neutron Dosimetry - MUNICH - Sept. 1974

(2) M. BRICKA, M. MOURGUES, L. PORTHEOS - Mesure des doses auprès de l'installation de neutronographie de Phénix - Rapport interne - Juin 1974

Résultats du projet nº 1

Chef du projet : Mr Michel MOURGUES

Titre du projet : Détermination des courbes de réponse des sphères de Bonner équipées de compteurs à hélium 3

Description des résultats :

La campagne de mesures effectuée, cette année, auprès du Van de Graaff du CEN/Cadarache a porté sur la bande d'énergies 10 keV - 2 MeV.

La figure 1 présente l'ensemble des résultats obtenus, dont on trouvera une description plus détaillée dans le rapport présenté à Munich (1). Ce document fixe, en particulier, les conditions d'étalonnage du matériel utilisé et les valeurs de référence :

- la source de référence est une source Am-Be de 1 curie émettant 2,62 10^6 neutrons par seconde dans 4π ,
- les mesures sont faites à 1,5 mètre au dessus du sol, avec une distance de 40 centimètres entre l'axe de la source et l'axe de la sphère.

Les valeurs de référence sont données dans le tableau ci dessous.

Diamètre de sphère	8"	10"	12"
Taux de comptage Impulsion/n.cm^{-2}	0,349	0,334	0,298

La concordance entre le calcul et la mesure est généralement très satisfaisante au dessous de 600 keV. Pour les énergies plus élevées, par contre, les courbes calculées par R. CAIZERGUES pour les sphères de 6" , 8" et 10" - en trait plein sur la figure - se placent nettement au dessus des valeurs mesurées. Cette divergence n'apparait pas pour les autres diamètres de sphères. Les résultats obtenus cette année confirment donc, sur ce point, les mesures faites antérieurement et la nécessité de campagnes de mesure supplémentaires pour les énergies supérieures à 600 keV.

La réponse aux neutrons thermiques, pour les différents diamètres de sphères, a été déterminée par des mesures auprès de l'empilement SIGMA. Les résultats (figure 2) recoupent de façon satisfaisante les calculs de R. CAIZERGUES et ceux de J. LAMBERIEUX.

Par contre, les premiers essais effectués pour obtenir les réponses à l'énergie de 0,4 eV, par la méthode de la fenêtre cadmium (différence entre deux écrans d'épaisseur 17/100 et 14/10) n'ont pas donné des valeurs convenables, le principal problème restant la mesure exacte de la fluence des neutrons correspondant à la fenêtre cadmium.

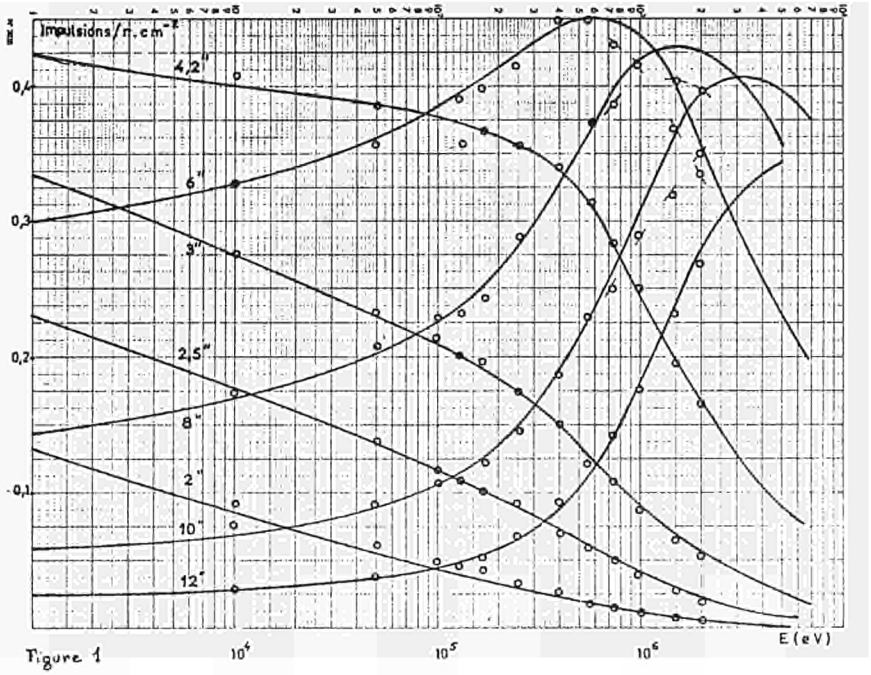

Figure 1

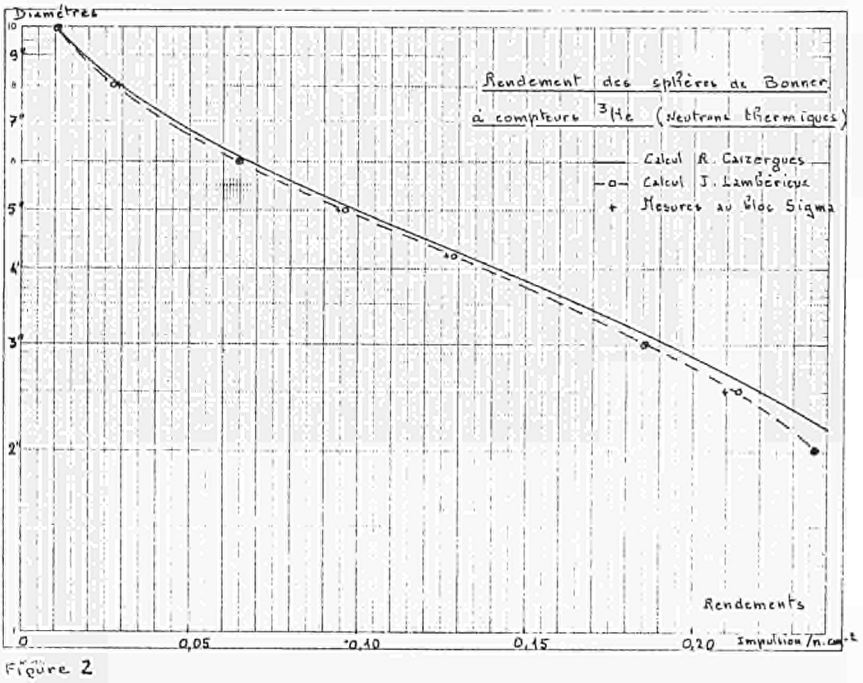

Figure 2

Résultats du projet n° 2

Chef du projet : Mr Michel BRICKA

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

Description des résultats :

La sonde de 10 pouces à compteur hélium 3 a été étalonnée, pour les mesures en doses, auprès de l'empilement SIGMA.

Pour un ensemble compteur-préamplificateur dont le comptage de référence est de 0,334 impulsion/n.cm^{-2}, la sensibilité est de :

$$8{,}0 \cdot 10^{-5} \text{ millirem par impulsion}$$

Une telle sonde, associée à un analyseur Spectrozoom travaillant en multiéchelle, a été utilisée pour des mesures en dynamique à la centrale Phénix. La figure 3 montre l'évolution, en fonction du temps, de la dose délivrée par une source solution travaillant en régime pulsé. La dose totale, intégrée au point de mesure était de 0,80 mrem.

Pour les mesures de fluence, la sensibilité de la sphère de 4,2 pouces varie, suivant l'allure du spectre, de 8 à 16 n.cm^{-2} par impulsion. On retiendra comme valeur moyenne :

$$12 \text{ n.cm}^{-2} \text{ par impulsion}$$

Il reste évident que ces deux sondes monosphères simples ne permettent pas d'obtenir des précisions meilleures que 25 à 30 %.

Les programmes de calcul SESR 1590 et SNAC 003 ont été aménagés pour traiter le système multisphère et un système réduit à 4 sphères. Ces programmes simples, basés sur la méthode des spectres modèles, permettent d'établir facilement des formules linéaires pour l'exploitation des données de mesure. On obtient ainsi :

- pour la fluence :

$$\Phi = (4{,}0\, N_{2,5"} - 4{,}8\, N_{2,5"/Cd} + 2{,}9\, N_{4,2"} + 1{,}6\, N_{10"})\ \text{n.cm}^{-2}$$

- pour le Kerma :

$$K = (7{,}8\, N_{2,5"} + 1{,}0\, N_{2,5"/Cd} - 18{,}3\, N_{4,2"} + 105\, N_{10"})10^{-10}\ \text{H.erg.g}^{-1}$$

- pour la dose équivalente :

$$D = (13\, N_{2,5"} - 1{,}9\, N_{2,5"/Cd} - 20\, N_{4,2"} + 119\, N_{10"})10^{-9}\ \text{rem}$$

Pour établir ces formules, on a utilisé les courbes de réponse Iodure de lithium affectées d'un coefficient 1,6. Elles seront, bien entendu, à revoir lorsqu'on disposera de données définitives pour les sphères équipées de compteurs hélium 3.

Pour les nouveaux détecteurs, les essais ont porté sur la modification de la réponse de la sphère de 10" par des perforations. Les résultats obtenus au Van de Graaff sont donnés sur la figure 4. Avec ces perforations et un taux de capture plus élevé dans le polyéthylène, il parait possible d'approcher la courbe de dose équivalente. L'aspect calcul de ce problème serait traité par Mr ROHLOFF, à Jülich.

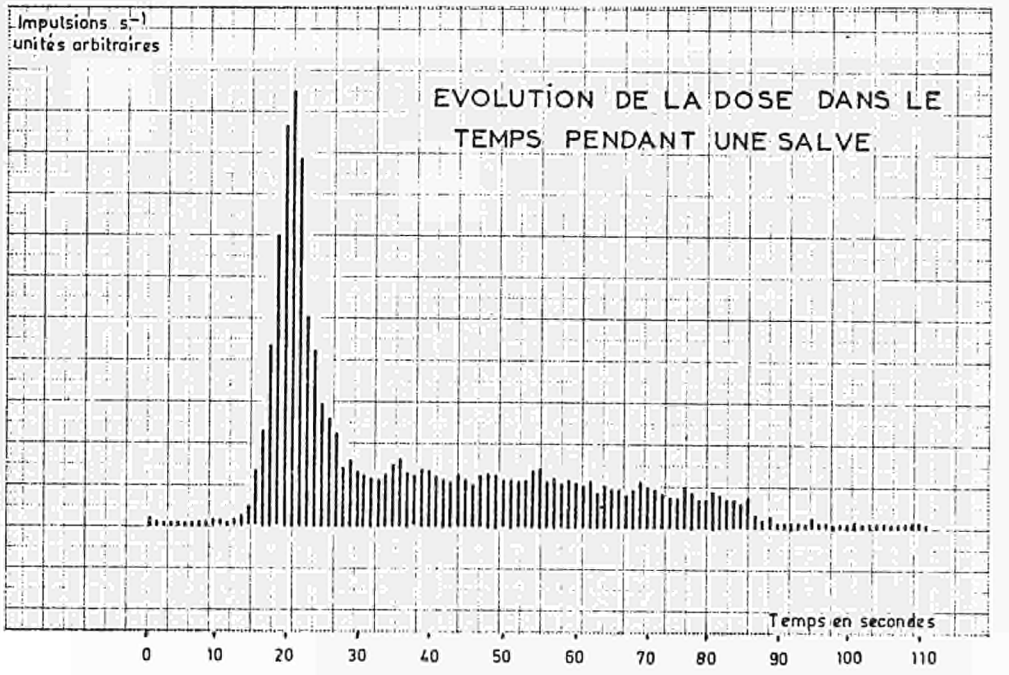

Figure 3

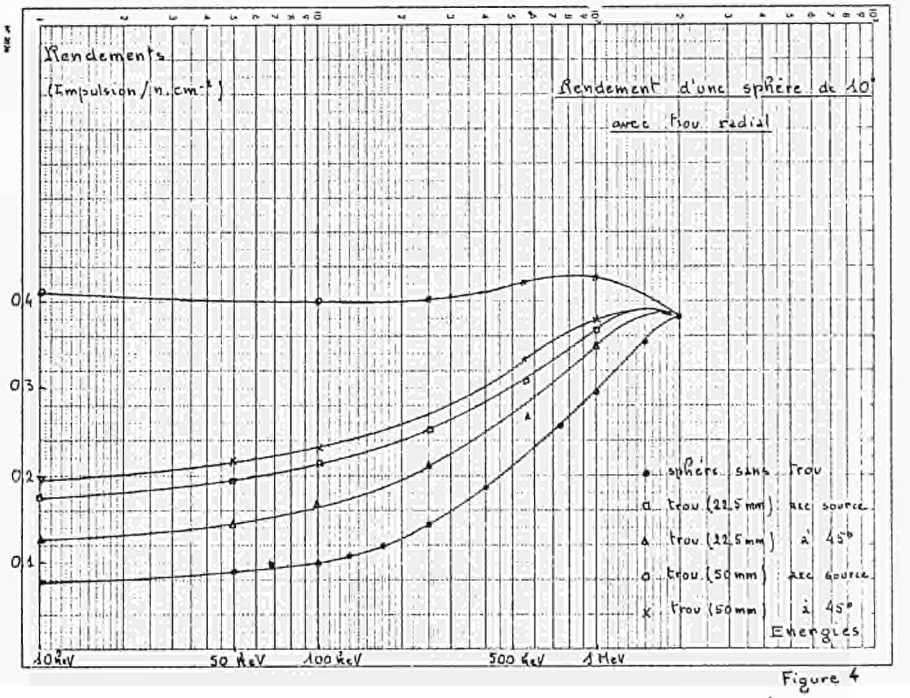

Figure 4

Kernforschungsanlage Jülich GmbH, Zentralabteilung Strahlenschutz, D 517 Jülich

Contract No. 065-72-1 PSTC

M. Heinzelmann

Neutron dosimetry with moderator spheres

Under the contract we investigate Bonner spheres for neutron dosimetry. There are two aims. First, we want to develop a light, energy - independent rem counter. Second, we study existent neutron dosimeters systematically in order to recognize error sources and their influence on detector readings.

This year we were especially successful in our first aim of developing a light energy - independent rem counter. We have constructed a polyethylene moderator with a ^{3}He-counting tube, clad in Cadmium. We use a suitably chosen discriminator threshold for the pulses of the ^{3}He-counting tube. We have shown that our device gives an energy independent dose rate equivalent.

We have developed a Monte-Carlo-Program in order to study the influence of the light-pipe on the efficiency of the moderating sphere counter. We have calculated the energy dependence for one moderator diameter and have found a good agreement with measurements at such energies where sources are available.

Results of the project:

Neutron dosimetry with moderator spheres

F. Rohloff and M. Heinzelmann

a) Low weight rem-counter

Such energy independent neutron dosimeters or so called rem counters as are available for practical health physics are of heavy weight and not easy to handle. We try to develop a low weight rem counter. For the purpose of health physics a rem counter may be considered reasonably energy-independent, when dose rate equivalent is given exactly within a factor of two in the energy range from 0,025 eV up to 10 MeV.

This year we have worked with a neutron dosimeter of following construction: A ^{3}He-proportional counting tube is clad with a Cadmium layer and surrounded by a spherical polyethylene moderator of 1,5" to 2" wall thickness. The ^{3}He counting tube gives pulses of different height due to the different energy of the reacting neutrons. At the capture of neutrons by ^{3}He an energy of 770 KeV will be released. Thermal neutrons will give a pulse spectra with a peak energy at 770 KeV. Fast neutrons will shift the peak of the spectra to higher energies, the peak will be the sum of 770 KeV plus the kinetic energy of the neutron. The discriminating threshold behind the ^{3}He counting tube is adjusted in such a way that only few pulses of the thermal spectra are counted and mainly those pulses which originated from fast neutrons. By changing the discriminator threshold the energy dependence of the dosimeter can be altered. In figure 1 we show this for the ^{3}He counting tube in a moderating sphere of 1,5". At a threshold of 975 mV - this is equivalent to a neutron kinetic energy of 55 keV - the sensitivity is quite independent from neutron energy. There are only few measurements with (α,n)-sources and the so-called "negative source" deviced by Mijnheer

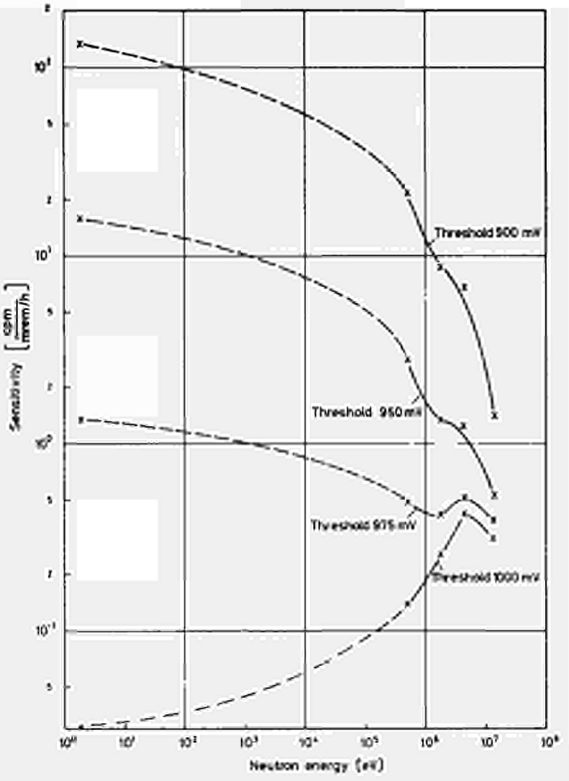

Fig.1: Variation of the energy dependency of efficiency from the discriminator threshold for a dosimeter consisting of a ^{3}He-counting tube and a polyethylene sphere of 1,5" wall thickness

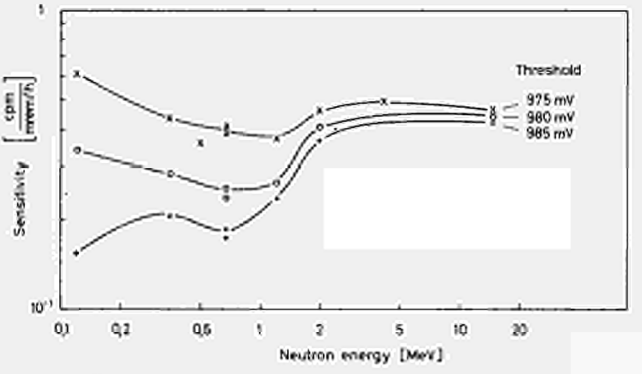

Fig.2: Efficiency of a ^{3}He-counting tube within a polyethylene sphere of 2" wall thickness for monoenergetic neutrons.

for the neutron energy of 1.8 eV. Some more exact measurements with monoenergetic neutrons have been done with the Van-de-Graaff generator of Neuherberg. They are shown in figure 2. Both figures prove the possibility of energy independent measurement of dose rate equivalent.

It is also possible to measure dose rate equivalent of thermal neutrons, if the Cadmium layer is perforated.

In comparison with other rem counters the new rem counter has the advantage of low weight and a range up to 15 MeV. His disadvantages are hitherto his low sensitivity, a pile up of single pulses with high dose-rates which give a reading that is too high, and the necessity of using a very stable electronic equipment. The sensitivity may be enlarged by a more sensitive ^{3}He counting tube and the pile up may be reduced by a faster electronic.

This principle for energy independent measurement of dose can be used for the construction of an albedo dosimeter. Preliminary experiments give reasonable hope that energy independent personal dosimetry may be possible with a small ^{3}He-counting tube and a polyethylene moderator of 0,5".

b) Dependency of ball counter sensitivity on the shape and material of the light pipe.

This year we have done calculations to determine the influence of the light pipe on the detector reading of Bonner spheres. Therefore a Monte-Carlo-Program was written that takes into account the spacial inhomogenity of the detector and the zylindrical light pipe. There is a great problem with thermal neutrons, which are not easy to handle with a Monte-Carlo-Program. Therefore we try to do the thermal calculation with a discrete-ordinate-program. With the full Monte-Carlo-Program we have calculated the efficiency of a 3" moderating sphere and a quartz light pipe. The calculations give a efficiency higher by 10 % than the earlier measurements indicate.

Publications:

M.Heinzelmann, F.Rohloff, H.Schüren, F.Sommer
Untersuchungen an Kugelmoderatordosimetern
in: Jül-1101-ST (1974) p. 204

M.Heinzelmann, H.J.Probst, H.Schüren
Bestimmung der Äquivalentdosis hochenergetischer Neutronen mit Spaltfragmentdosimetern hinter einer Zyklotronabschirmung
in: Jül-1101-ST (1974) p. 213

Apply for a patent:

M.Heinzelmann, F.Rohloff, H.Schüren
Gerät zur Bestimmung der Äquivalentdosis von Neutronen
Deutsche Patentanmeldung P 24 47 817.9

GESELLSCHAFT FÜR STRAHLEN- UND UMWELTFORSCHUNG MBH, MÜNCHEN
Institut für Strahlenschutz, Neuherberg

Vertrag Nr.: 065 PSTC

Leiter der Forschungsgruppe:
Dr. G. Burger, Prof. Dr. F. Wachsmann

Allgemeines Thema des Vertrages:
Personendosimetrie und Kalibriertechnik im Neutronenstrahlenschutz

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The main topics of the neutron dosimetry group are investigations on

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

24 men-months are planned for the contract, 12 men-months for neutron spectrometry and absolute source strength determination by bath methods, the rest split into activities of albedo dosimeter and remcounter calculations and calibrations with monoenergetic neutrons at the 3 MeV-Van De Graaff accelerator of the GSF.

References:

Alves, R.N.; D.C.C. Reis; S.M. Fernandes; G. Burger and F. Grünauer
The Use of Small $MnSO_4$-Baths for Calibrations of Neutron Sources
2. Symp. Neutron Dosimetry Biol.Med., Neuherberg, 1974

Ergebnisse des Projekts

Leiter des Projekts und wissenschaftliche Mitarbeiter:

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

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

1. Personel Dosimetry.

The albedo dosimetry was further investigated. The main problems with those dosimeters are their response functions with respect to neutron energy and the angle of incidence. There was designed and constructed an albedo dosimeter consisting essentially of a semisphere of polythene (diameter = 3.4 cm) with an external borated shield and a Li^6/Li^7 detector. In continuing the phantom radiation transport calculations, resulting in the spectral albedo flux densities, reported last year, the response function of this particular instrument was now determined on the basis of adjoint calculations. The human body was approximated by a cylinder the height of which was 20 cm and the diameter 40 cm. The dosimeter was positioned at the cylinder axis 0.5 cm distant from the top surface. The $Li^6(n,\alpha)$ cross section was used as the adjoint source spectrum; the source being homogeniously distributed throughout the Li^6 detector and from this the double differential adjoint neutron flux density emerging from the phantom surface was calculated. From this the response function of the dosimeter for every desired irradiation geometry can be gained.

The prototype with a borated shield was investigated theoretically and experimentally with monochromatic neutrons above 100 keV. There occured discrepancies, which could not yet be explained.(fig. 1).

The transport programs have further been used for the determination of the response-functions of moderator counters. The same counter was also checked experimentally with monoenergetic neutrons above 100 keV. The results have not yet been analyzed.

2. Calibration Techniques.

In the field of neutron standardization and calibration, the work in connection with the spectrometry of radionuclide sources, especially in the intermediate region, was continued. The same holds for the dosimetry and fluence measurements at the accelerators.

Some specific effort was given to absolute source strength determination by means of the $MnSo_4$-bath method. The standard techniques, using large bathes of generally more than 400 liters of solution seem to be well established.
The main disadvantage of the large bathes is however their low sensitivity. This is crucial for either the calibration of radionuclide neutron sources with low source strength, or accelerator target-reactions, where the irradiation time is always limited. In such cases the high random errors may be decreased by using smaller bath sizes with increased sensitivity.

In this case the neutron leakage q_1 is however increased, and the response function f(E) of the bath is no longer flat, but

$$f = (1 + \frac{C_H \; H}{C_{Mn} \; Mn}) \; / \; (1 - \frac{q_1 (E)}{Q})$$

(C = concentrations, Q = source strength).

To determine q_1 (E), calculations have been performed on the basis of the codes ANISN for spherical and DØT for cylindrical geometries, for a series of different radionuclide sources.

Figure 2 shows the situation for a spherical bath with 20 cm radius for an AmBe-radionuclide source.

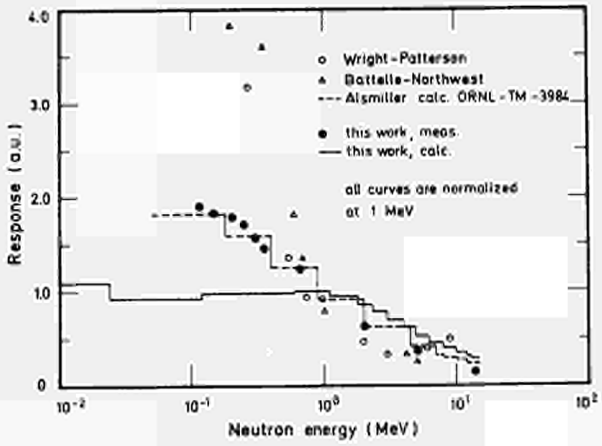

Figure 1:

Theoretical and experimental responses for two similar Albedo-dosimeters (of J.E. Hoy, DP-Rept. 1277 and own development)

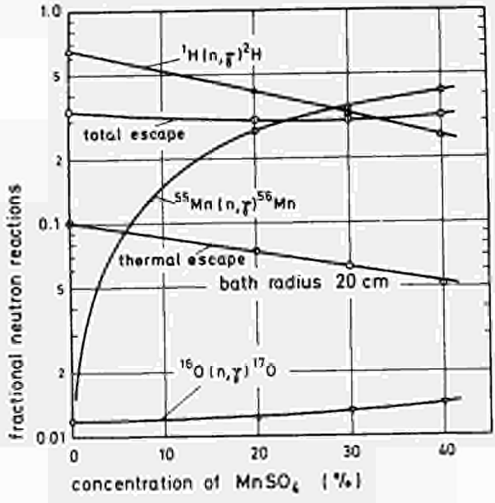

Figure 2:

Fractional reaction rates as a function of $MnSO_4$-concentration in a spherical bath with 20 cm radius

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

N° du contrat : 069 73 1 PST F

Chef du groupe de recherche : D. BLANC

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

Description générale succincte des travaux réalisés

Nos échantillons sont des verres de composition métaphosphatique ($M_2O/P_2O_5=1$).

Selon les natures et l'importance des éléments modificateurs nous avons quatre catégories de verres :

Pourcentage en mole	$NaPO_3$	$Ba(PO_3)_2$	$AgPO_3$
	88 %	12 %	0 %
	85, 7 %	11, 6 %	2, 7 %
	83, 2 %	11, 3 %	5, 5 %
	80, 7 %	11 %	8, 3 %

En continuation de l'étude actuellement en cours sur les propriétés électriques de ces verres non irradiés, nous poursuivons celle de leur comportement sous irradiation : modification de leurs caractéristiques en fonction du flux d'irradiation électromagnétique et de l'énergie déposée.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques :

J. BARTHE, L. COMMANAY, J. CASANOVAS.

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

Description des résultats

1 - Etude expérimentale du courant de conduction naturelle i_o

Etant donné les domaines limités de champs électriques et de températures où nous travaillons, les relations i_o fonction linéaires et exponentielles de E sont observées. Mais les résultats s'accordent mieux avec un loi du type $i_o \sim \exp \sqrt{E}$ qu'avec $\sigma \sim \exp \sqrt{E}$ aux champs les plus élevés.

Les énergies d'activation de cette conduction calculée expérimentalement à 10^5 V/cm sont de 5 % supérieures à celles obtenues à 10^4 V/cm (rapport Euratom 73).

2 - Etude du courant induit i_i par rayonnement gamma du du ^{60}Co

Les courbes $i_i(E)$ ont la même allure que celles obtenues pour les diélectriques liquides - pas de courant de saturation, le palier incliné (pente=0, 1 à 0, 2 pA/cm. V) est suivi aux forts champs électriques d'une croissance exponentielle.

Les résultats obtenus en fonction du débit de dose $\frac{dD}{dt}$ quand la cellule est isolée thermiquement sont bien représentés par la relation $i_i = K \left(\frac{dD}{dt}\right)^{\Delta}$.

Selon la composition du verre, on observe des valeurs différentes,

- pour l'exposant Δ :

$\Delta \leq$ 0, 5 verre sans argent

$\Delta \geq$ 0, 5 à 1 verre avec argent

- pour les coefficients de température de cette conduction induite :

$E_i \simeq 0,075$ eV verre sans argent

$E_i \simeq 0,003$ eV verre avec argent

En conclusion :

Avec une résistivité intrinsèque de 10^{11} à 10^{13} Ω . cm à 20°C, nos échantillons sont relativement isolants.

La conduction induite apparaît notable, supérieure à 1n A/cm^2 à partir de débit de dose de 50 à 100 rads/heure.

L'introduction d'argent se traduit par une inhibition du courant naturelle i_o et par un effet de piégeage électronique sous irradiation.

Nous n'avons quasiment pas observé de variation de la résistivité intrinsèque avec la dose intégrée jusqu'à 100 krads.

La figure 1 donne i_o et i_i à 3000 rads/h en fonction de l'inverse de la température entre 10 et 80 KV/cm.

PUBLICATIONS

Contribution à l'étude des conductions naturelle et induite par rayonnement gamma du cobalt 60 des verres aux métaphosphates activés à l'argent.
VELOMPANAHY (A.) : Thèse de doctorat de 3° cycle, n°1695 TOULOUSE, 17 Janvier 1975.

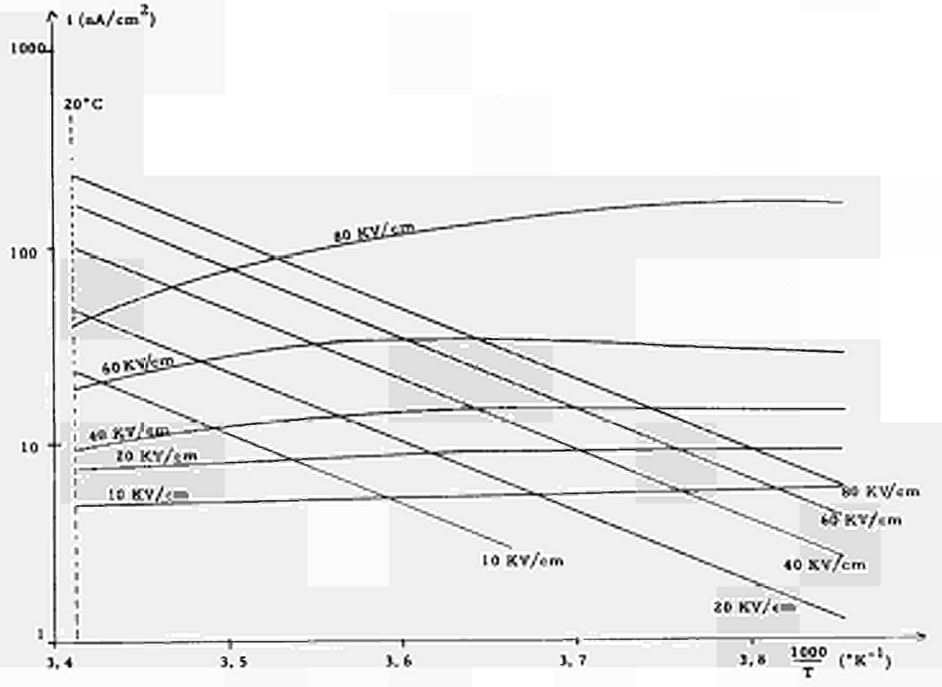

Figure 1 - Courant naturel i_o et induit i_i en fonction de $\frac{1000}{T}$ entre 10 et 80 KV/cm.

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

Contract No.: 074-74-1 PSTUK

Head of research team: D.H. Peirson

General subject of contract: PASSIVE DOSIMETRY

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

Project 1. Passive detectors for neutron dosimetry and spectrometry

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

Project 2. Dosimetry in mixed radiation fields

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

Results of Project No. 1

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

Title of Project: PASSIVE DETECTORS FOR NEUTRON DOSIMETRY AND SPECTROMETRY

Development of a personal neutron dosimeter based upon neptunium-237 fission

Neptunium-237 fission foils (4 mg) have been used with polycarbonate films to produce a detector for fast neutrons (> 0.5 MeV) to supplement the albedo dosimeter discussed in project 2. Fission tracks in the polycarbonate film are etched in hot caustic potash (KOH) solution and counted with a spark counter. The dosimeter has been calibrated with a range of neutron energies as discussed in project 2. The response is such that 1 count is equivalent to about 3 mrem and a possible reporting level of 50 mrem is easily detectable. The dosimeter and spark counter are being developed for routine use in a fuel reprocessing plant and a report on the work is being prepared.

A review of activation and fission detectors

A review of activation and fission detectors for neutron flux and spectrum determination has been prepared. It covers the use of these detectors in reactor cores and in shielding studies, as well as in dosimetry investigations, and includes a review of the mathematical methods of treating the data.

Some consideration was given to the use of resonance detectors for dosimetry investigations, but as a result of the review it was concluded that the most fruitful course to follow for radiological protection investigations is the use of moderating spheres. Accordingly, a set of such spheres from 5 cm to 25 cm diameter has been designed and manufactured. They will be used initially with a LiI scintillator and later with uranium-235 fission foils. A careful investigation of the response functions will be made, particularly in the 1-1000 keV region, using monoenergetic neutrons from the 3 MeV Van de Graaff accelerator IBIS.

The moderating spheres will be used in conjunction with the existing

threshold detector system, to supply information on the neutron flux in the region from 1 keV to 500 keV.

Threshold detector system for neutron spectrometry

The standard system consists of a sulphur disc, gold foils and fission foils of uranium-238, neptunium-237, uranium-235 under boron, uranium-235 under cadmium, uranium-235 bare. The fission tracks are recorded in polycarbonate film and counted optically. Investigations are sometimes made with a simplified system consisting of sulphur, neptunium-237 and gold. Several systems are available and they are particularly useful for investigations of the spatial variation of the field.

The standard system is being used in the determination of the neutron spectrum obtained from the VIPER reactor (AWRE Aldermaston) which is to be used for the Fourth IAEA Intercomparison of Nuclear Accident Dosimetry Systems in April 1975. The spectrum can be measured using counters when the reactor is run in the steady state but for the intercomparison the reactor will be run in the pulsed mode, and the threshold system is being used to make sure that the spectra in the two modes of operation are the same. The simplified threshold systems have been used to investigate the uniformity of the field.

Results of Project No. 2

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

Title of Project: DOSIMETRY IN MIXED RADIATION FIELDS

Comparison of albedo dosimeters developed in the UK and Germany

In order to choose a suitable albedo dosimeter for use in a fuel reprocessing plant we have determined the response of dosimeters from several European laboratories to monoenergetic neutrons in the energy range 0.1 MeV to 1.7 MeV. Dosimeters were supplied by Brunskill (BNFL, Windscale), Berger (GSF, Munich), Harvey (CEGB, Berkeley), Piesch (GKF, Frankfurt) and Preston (AEEW, Winfrith). All the dosimeters except Brunskill's were based upon pairs of TLD chips of ^{6}LiF and ^{7}LiF. Brunskill used natural lithium borate as a thermal neutron detector.

Monoenergetic neutrons of 0.1 MeV to 1.7 MeV were produced by photon reactions on lithium and tritium with the 3 MeV Van de Graaff accelerator IBIS, at Harwell. The dosimeters were also irradiated with a californium-252 source of fission neutrons and thermal neutrons from the GLEEP reactor. The irradiations were performed in essentially scatter-free conditions at 5 m from the floor in a very large room. A Harwell long counter was used for calibrations. The effects of residual scatter was determined at two energies by using a shadow cone. Preliminary results have been obtained and a complete analysis is in progress.

Effect of annealing position on the sensitivity of LiF chips

In an attempt to reduce the variation in sensitivity of LiF chips in a batch, the effect of position in the annealing oven and possible variations in the calibration dose were investigated. No significant variations in the calibration dose were observed but the effect of position in the oven was significant (> 5% variation) and reproducible. Chips are now annealed in small batches in an area which gives uniform sensitivity and methods of obtaining a more constant temperature over a wider area are being investigated.

Light sensitivity of LiF-PTFE discs

Two separate batches of thin discs (~ 9 mg cm^{-2}) have shown light sensitivity. One batch was light sensitive on arrival and an earlier batch suddenly became light sensitive after 16 anneals. The annealing cycle is normally 25 min at 300°C and 16 h at 80°C but on several occasions 1 h at 400°C and 16 h at 90°C has been used. The apparent dose produced can be equivalent to several hundred millirads. Discs are less sensitive to light of wavelength above 600 nm than from 400 to 600 nm. Possible reasons for the light sensitivity are being investigated.

Neutron effects in gamma ray dosimetry with LiF-PTFE discs

The two effects studied so far have been the direct response to fast neutrons and the indirect response to thermal neutron induced reactions in activation foils in the UK Criticality Locket. The direct response to fast neutrons is 1.2×10^{-10} gamma rad equivalent per neutron cm^{-2} (gamma rad n^{-1} cm^2) or about 0.03 gamma rad per neutron rad in good agreement with other published data. The direct effect of thermal neutrons is of the order of 5×10^{-11} gamma rad n^{-1} cm^2 but in the locket (n,γ) reactions produce about 6×10^{-10} gamma rad n^{-1} cm^2. This response is due mainly to cadmium (70%) with the remainder due to the indium foil. The effects of gold foils is negligible. Further investigations are continuing.

Derived working limits for surface contamination by specific isotopes

Derived working limits (DWL's) for surface contamination have been calculated for specific nuclides by calculating the dose under the epidermal layer from a plane source of the nuclide. This has involved calculations for electrons, beta and gamma radiation with some preliminary calculations for alpha radiation. Point source functions for each radiation have been developed from various sources. It has been shown[1] that generalised DWL's based upon strontium-90 and lead-210 are overly restrictive by factors up to 100 for some nuclides.

Electrets as radiation dosimeters

Studies of the effects of radiation on electrets have shown that the discharge of the electret is due to ionisation in the surrounding air and not to the direct effects in the material. Electrets are therefore unlikely to be useful as radiation dosimeters for personnel use and a report on the work is being prepared.

Reference

(1) GIBSON, J.A.B., WEBB, G.A.M., WRIXON, A.D. A comparison of general and specific derived working limits for surface contamination with reference to low toxicity isotopes. International symposium on radiation protection, Aviemore, Scotland, 2-4 June 1974.

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

The basic principles of the design of a neutron monitor were studied with a view to defining possible instrumental systems. Through personal contacts and literature survey we took account of related studies at other laboratories. Having identified the basis of two possible instrumental designs we studied the physics of the interaction of electrons and protons with appropriate gaseous and scintillation detectors in order to define the required characteristics of the detector.

The work to date has not been formally reported.

Results of Project No. :	078-74-7 PSTUK
Head of Project and scientific staff :	J.R. Harvey. (0.3 from 1st July 1974).
Title of Project :	Feasibility assessment of a low weight wide range rem-response survey instrument.

Our studies suggest that a two component system could, in principle, be devised which would have low weight and wide range. The two components would be sensitive to high and low energy neutrons with a take-over point at around 10 keV. A major problem is in defining a component sensitive to high energy neutrons with a threshold as low as 10 keV. A number of groups at other laboratories have studied two component systems but in all cases these suffer from the disadvantage that the low energy threshold of the high energy component is 100 keV or more whereas the upper threshold of the low energy component is about 10 keV. The instruments, therefore, are insensitive within the particularly important energy range; 10 keV - 100 keV.

Theoretical considerations suggest that sensitivity down to 10 keV is in principle obtainable by using a detector in which the sensitive volume has very low weight per unit area.

Two systems were identified as worth further consideration. The first is a proportional counter with linear dimensions of the order of 1 cm operating at gas pressures around 10 torr. Such a system would suffer from the disadvantage of low sensitivity so that readout would have to be by digital integration. The second system would utilize a scintillator with a thickness around 1000A. If such a scintillator could be produced with an area of about 100 cm^2 the resulting device would have sufficient sensitivity to operate a ratemeter.

Either of these systems could be used in conjunction with a thermal neutron detector inside a 4" diameter moderating assembly to give rem-response from thermal energies to 10 MeV or more. Both systems will be the subject of further study.

TRANSPORT VON RADIONUKLIDEN IN DEN KOMPONENTEN DER UMWELT

TRANSFER OF RADIOACTIVE NUCLIDES IN THE CONSTITUENTS OF THE ENVIRONMENT

CHEMINEMENT ET TRANSFERT DES RADIONUCLIDES DANS LES COMPOSANTS DU MILIEU AMBIANT

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

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

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

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

Biology Group Ispra

Contractant de la Commission :
Commissariat à l'Energie Atomique - Fontenay-aux-Roses, France

N° du contrat : 061-72-1

Chef du Groupe de Recherche : G. LACOURLY

Thème général du contrat : Niveaux de Pollution du milieu ambiant -

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L'objet du contrat consiste à rassembler et, éventuellement, élaborer les données et méthodes nécessaires pour évaluer, en tout point du territoire de la Communauté Européenne, et en fonction des paramètres qui déterminent les caractéristiques locales, les limites acceptables de la pollution radioactive du milieu ambiant et de la chaîne alimentaire.

Cinq projets principaux sont en cours :

1. Etude des paramètres biologiques de l'homme européen.

2. Etude des paramètres de la contamination de l'environnement à partir de la pollution de l'atmosphère.

3. Etude des paramètres de transfert à l'homme de la contamination à partir de la pollution des eaux et au cours de la préparation des aliments de l'homme à partir des produits bruts.

4. Etude des transferts de la pollution à partir des sédiments et des sols.

5. Etude des problèmes posés par la dispersion et la dilution de la contamination radioactive dans les circuits de distribution des produits alimentaires.

En outre, des études d'orientation portant sur l'évaluation de la dose collective à l'échelle régionale ont été entreprises.

Résultats du projet N° I -

Chef du projet et collaborateurss scientifiques : Dr L. KARHAUSEN

Titre du projet : Etude des paramètres biologiques de l'homme européen -

Description des résultats :

1. Thyroïde - Enquête européenne -

L'étude de la captation thyroïdienne à 24 heures dans les six pays s'est poursuivie. Le but de cette seconde étape est double : rassembler des résultats dans les zones d'ombre où la première enquête n'avait pas récolté de données et plus particulièrement dans les régions où l'on a des raisons de croire qu'il pourrait s'agir de valeurs extrêmes (par exemple, dans les régions où le goître est répandu).

Le second but est de standardiser les valeurs obtenues au cours de l'enquête. Cette seconde phase a été quelque peu ralentie du fait de difficultés techniques. Un mannequin déjà utilisé par l'IAEA devra circuler dans les divers centres qui ont collaboré au programme. Les difficultés proviennent du problème de l'obtention d'un mélange stable et homogène de résines et d'isotopes qui fournissent un pic comparable à celui de l'iode 131. Ce problème a été résolu au cours de l'année écoulée et l'épreuve de standardisation des mesures relevées dans les différents laboratoires est commencée.

2. Métabolisme du cadmium et du mercure -

La communication faite en 1973 sur l'absorption intestinale du cadmium et du mercure a fait l'objet d'une publication dans un volume de la Commission Européenne.

De plus, un travail a été rédigé en collaboration avec M. Magnaval sur les risques professionnels du mercure utilisé dans les cabinets dentaires.

3. Homme standard -

Le texte de travail sur l'Homme standard rédigé en collaboration avec l'ICRP est sous presse chez l'éditeur Pergamon. Par ailleurs, un programme a débuté vers la fin de l'année pour l'étude de la composition minérale du corps humain. Ce travail est en cours à l'Institut de Médecine Légale de la Faculté de Médecine de l'Université de Lübeck (Professeur Pribilla).

Les premiers résultats ont été obtenus sur la composition minérale du squelette et ils seront ensuite étendus aux tissus mous.

Résultats du projet N° 2 -

Chef du projet et collaborateurs scientifiques : L. ANGELETTI

Titre du projet : Etude des paramètres de la contamination de l'environnement à partir de la pollution de l'atmosphère -

RESULTATS

1. Etude de la contamination foliaire par voie humide -
(collaboration de la Division de Biologie à Ispra)

Les résultats des études effectuées en 1973 ont été élaborés au cours de 1974 et font l'objet d'un rapport actuellement sous presse.

Le programme 1974 concernant la rétention, le lessivage et l'accumulation de l'iode et du strontium sur les parties aériennes du ray-grass et du trèfle a été achevé. Les principaux résultats sont les suivants :

- Période biologique de l'iode et du strontium.-

Les valeurs moyennes trouvées sont comprises dans l'intervalle 13-22 jours et concordent avec celles reportées dans la littérature.

- Lessivage et accumulation -

Ces deux phénomènes semblent dépendre tant de la nature de l'élément chimique que de celle du végétal.

On observe, en effet, que, en ce qui concerne l'accumulation, l'iode ajouté aux végétaux 24 h après le premier traitement ne modifie pas pour le ray-grass la concentration initiale, alors que pour le trèfle, l'augmentation est significative ; pour le strontium, l'accumulation est toujours vérifiée sur les deux espèces végétales.

En ce qui concerne le lessivage effectué avec de l'eau 24 h après le dépôt des radionucléides, on observe que l'eau élimine environ 50% de l'iode présent sur les feuilles du ray-grass et du trèfle et environ le même pourcentage du strontium sur le ray-grass lorsque son effet est nul sur le strontium sur le trèfle.

2. Etude du dépôt et de la rétention des métaux lourds sur l'herbe-
(collaboration du K.F.A., Jülich)

L'étude du dépôt des aérosols des métaux lourds sur la végétation se déroule normalement et devra être achevée au cours de l'année 1975.

Les travaux effectués à ce jour portent sur la mise au point du protocole expérimental ainsi qu'à la mise au point des systèmes de production des aérosols et à la mesure de leurs caractéristiques physico-chimiques. L'étude sera poursuivie avec des aérosols de sulfate de cuivre radioactifs, qui, grâce à la facilité de leur détection pourra permettre un nombre de mesures très élevé et ainsi servir de référence, puis avec les composés du plomb tels qu'ils sont produits dans un moteur d'automobile.

Les essais " in situ " sont actuellement en cous d'éxécution.

3. Etude bibliographique du plutonium dans l'environnement et chez l'Homme -

Une étude bibliographique est en cours et elle fera l'objet d'une publication prochaine.

Résultats du projet N° 3 -

Chef du projet et collaborateurs scientifiques : R. BITTEL, R. MAGNAVAL, Mme A. GARNIER

Titre du projet : Etude des paramètres des transferts à l'Homme de la contamination à partir de la pollution des eaux et au cours de la préparation des aliments de l'Homme à partir des produits bruts -

RESULTATS

1. Etude de la contamination des végétaux irrigués (collaboration de la Division de Biologie d'Euratom à Ispra et du Laboratoire de Radioécologie du DPr, à Cadarache)

- Contamination des végétaux irrigués par submersion -

L'étude de la contamination des rizières et du riz en installation pilote a été poursuivie. Les éléments étudiés sont, d'une part, le zinc 65 et, d'autre part, le cadmium stable. Le problème de la microlocalisation de ces deux éléments dans les divers organes de la plante est actuellement envisagé.

- Contamination des végétaux irrigués par aspersion -

On a étudié notamment la contamination du maïs-grain par le zinc 65, les paramètres étant les concentrations en cadmium et zinc stable des eaux d'irrigation. En l'absence de Cd et de Zn volontairement ajoutés, les facteurs de transfert du zinc 65 eau d'irrigation --→ grains sont de l'ordre de 5, ils sont abaissés en présence de cadmium et de zinc stable.

2. Contamination des organismes dulçaquicoles -

- Contamination des poissons par le zinc 65 -

Deux recherches sont en cours, l'une à Ispra, l'autre à Cadarache, les paramètres envisagés étant les teneurs des eaux en cadmium et en zinc stable.

- Contamination d'un écosystème naturel par le tritium -

Cette recherche, en collaboration avec le Département de Radiobiologie du CEN belge de Mol a en particulier montré que, dans le cas d'écosystèmes naturels recevant des effluents liquides tritiés, l'action spécifique de l'hydrogène de la matière organique des végétaux et animaux récoltés était supérieure (dans un rapport de 1 à 10 ou 100) à celle de

l'hydrogène de l'eau. Cette étude doit être poursuivie dans le cadre de nouvelles recherches sur les radionucléides à diffusion mondiale.

3. Contamination des chaînes trophodynamiques marines -

On a entrepris l'étude de la dernière chaîne biologique prévue dans le programme général , celle d'une chaîne de type benthique à crustacés (eau - bactéries marines, annélide, crustacés). Les éléments étudiés sont comme précédemment le cuivre, le zinc, le chrome ($Cr^{(VI)}$), le plomb et le mercure. Les premiers résultats montrent l'existence de phénomènes de concentration, notamment dans les premiers échelons trophiques. Les renseignements obtenus pour les trois premières chaînes sont en cours d'interprétation, notamment en ce qui concerne la microlocalisation des polluants dans les organismes aquatiques.

4. Transfert des polluants des produits bruts aux produits alimentaires au stade de leur consommation par l'Homme -

En collaboration avec le Laboratoire de Biologie Végétale du Département de Recherche Fondamentale du CEA et le Centre de Brasserie-Malterie de l'Université de Nancy, on a suivi le transfert d'éléments métalliques (Sr, Cd, Pb, Hg, Cr, Co, Cu, Fe, Mn, Mo) des produits utilisés dans la fabrication de la bière à la bière elle-même. On constate une élimination progressive dans tous ces éléments qu'on retrouve en fortes concentrations dans les drêches. Une étude sur le transfert des métaux lourds dans les produits céréaliers est actuellement débutée en relation avec l'Ecole Française de Meunerie et l'INRA.

5. Etudes d'orientation -

Des études prospectives ont débuté, en vue de préciser le programme ultérieur de recherches. Les principaux points envisagés sont :

- l'utilisation de chaînes trophiques marines naturelles ou artificielles pour l'étude des transferts du plutonium, du tritium et de l'iode 129,

- les problèmes de microlocalisation des radionucléides en fonction de leur état physico-chimique, de l'existence de pollutions associées et de rejets thermiques, en vue d'apprécier les effets de faibles doses sur le milieu,

- les problèmes posés par les diverses utilisations des déchets industriels, agricoles et urbains.

Résultats du projet N° 4 -

Chef du projet et collaborateurs scientifiques : R. MAGNAVAL, R. BITTEL

Titre du projet : Etude des transferts de la pollution à partir des sédiments et des sols -

RESULTATS

1. Détermination du méthyl-mercure et du sélénium dans les échantillons biologiques et les sédiments (collaboration avec le Laboratoire de Pathologie et Toxicologie Expérimentale du C.E.A.) -

La méthode de dosage reprend essentiellement la procédure d'analyse déjà publiée au Colloque Européen de Luxembourg. L'étape de minéralisation a été plus particulièrement étudiée afin de limiter la solubilisation dans la phase aqueuse du mono et du diméthyl-mercure lorsque ces sels sont en présence d'un excès d'ions chlorures et permettre une extraction quantitative par le benzène. Le temps optimum de contact de l'acide chlorhydrique est de l'ordre de quatre heures. Dans 15% des échantillons de sédiments prélevés dans la région minière de Monte-Amiata (Italie), on observe la présence de méthyl-mercure. La fraction méthylée est inférieure à 0,06% pour des sédiments contenant 10 à 150 ppm de mercure. Dans les échantillons de végétaux aquatiques non lavés, associés à des matières en suspension du cours d'eau, la fraction méthylée est de 0,13 à 0,27%.

La détermination du sélénium dans les échantillons biologiques est effectuée par complexation du sélénium avec le diaminonaphtalène et extraction du complexe par l'hexane. Cette méthode est en cours de mise au point.

2. Une étude bibliographique a d'autre part été entreprise pour évaluer la résistance de certains microorganismes à des composés en concentration normalement toxiques dans le milieu. Ces facteurs de résistance jouent un rôle important dans la méthylation ou la diméthylation du mercure et permettent d'expliquer en partie les résultats précédemment obtenus sur des sédiments prélevés in situ.

3. Fixation du mercure sur des sédiments en fonction de la salinité de l'eau (collaboration aux travaux du Laboratoire de Biologie Végétale du CEN-Grenoble) -

$$L'équation\ empirique\ C = Co\ \left(1 + \frac{t}{a} \right)^{n}$$

traduisant la cinétique d'épuration d'une eau de surface s'est

déjà avérée satisfaisante dans le cas de nombreux radio-éléments introduits dans des écosystèmes simplifiés très divers.

La valeur moyenne des paramètres "a" et "n" est donnée, après ajustement par la méthode des moindres carrés, pour le chlorure mercurique (a = 9,2, n = 1,1) et pour le chlorure de méthyl-mercure (a = 2,9 - n = 1,2). Lorsque l'on introduit du chlorure mercurique et du chlorure de méthyl-mercure (^{203}Hg) dans des écosystèmes comportant de l'eau et des sédiments avec des teneurs en sels comparables à celles d'un estuaire, les paramètres "a" et "n" varient linéairement avec la racine carrée de la salinité. Dans ces mêmes conditions expérimentales, on obtient, à l'équilibre, les valeurs des coefficients de distribution. Une salinité de l'eau de 35‰ diminue la valeur du coefficient de distribution dans les sédiments de 60 à 80%.

4. Migration de l'iode dans le sol (collaboration avec la Division de Biologie d'Ispra)

L'étude a débuté par l'évaluation de la volatilisation de l'iode déposé à la surface du sol, préalable indispensable, avant de considérer sa migration dans les couches profondes. Cette volatilisation a été étudiée sur des disques calibrés de papier filtre sur lesquels une solution d'iodure de sodium marquée à l'iode 131 a été appliquée.

Dans les conditions expérimentales et pour une luminosité et un pH proches des conditions naturelles, on peut estimer que la volatilisation est comprise entre 5 et 10% de l'iode déposé pendant les premières vingt quatre heures.

L'étude de la migration proprement dite de l'iode dans le sol est en cours.

Résultats du projet N° 5 -

Chef du projet et collaborateurs scientifiques : Mme A. GARNIER

Titre du projet : Etude des problèmes posés par la dispersion et la dilution de la contamination radioactive dans les circuits de distribution des produits alimentaires -

RESULTATS

L'étude a porté sur les problèmes concernant les produits laitiers.

Le facteur de dilution, d_i, relatif à un produit laitier quelconque i peut être défini comme étant le rapport entre les concentrations dans ce produit :

- soit après dilution du lait, transformation en produit commercial, et mélange à d'autres produits du même type, mais de provenances différentes (concentration C_{id}),

- soit par fabrication et consommation sur place (C_{ip}),

d et p : relatifs respectivement aux centres de distribution commerciale et de production agricole.

On peut définir de façon analogue le facteur de dilution se rapportant à l'ensemble de la ration alimentaire en produits laitiers.

Le facteur de dilution dépend des paramètres suivants :

- productions de lait des différentes régions
- proportions collectées de chacune d'elles par les organismes collecteurs situés dans les autres
- concentrations des éléments dans le lait entier au stade de la production laitière
- facteurs intervenant au niveau de la transformation industrielle : utilisations du lait entier, rendements de transformation, facteurs de concentration au cours de la transformation

- facteurs intervenant au stade de la commercialisation, circuits de distribution, susceptibles de variation dans le temps en fonction des réorganisations dans l'industrie laitière ou de l'évolution des marchés

- enfin, en ce qui concerne la dilution de l'ensemble de la ration en produits laitiers : composition de cette ration selon l'âge et les habitudes alimentaires.

La formulation du problème est relativement simple mais sa résolution nécessite de nombreuses données.

La complexité des circuits de distribution peut s'ajouter pour la catégorie des fromages (autres que les produits frais), à celle des circuits de fabrication. On peut alors admettre que la contamination moyenne dépend uniquement de la contamination moyenne des laits utilisés et du facteur de concentration approprié.

En conclusion, de nombreux problèmes pratiques se posent dans une évaluation de ce type, qui peut être cependant justifiée en cas de contaminations très élevées en certaines régions par rapport au niveau moyen.

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Secrétaire	M. BRESSON
Membres	Dr RECHT
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Etudes atmosphériques	L. ANGELETTI
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Documentation	R. HAMMER
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Contract No. 104-72-1 BIAI

Laboratorio per lo Studio della Contaminazione Radioattiva del Mare
Association CNEN-EURATOM - Fiascherino (La Spezia) Italy

Dr. Michael Bernhard

Title:

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

The projects of the association are organised according to the different levels of the food chain; physical and chemical environmental factors, first trophic levels (phytoplankton), first heterotrophic levels (zooplankton and bacteria), last heterotrophic levels (crustaceans and fish). Unfortunately one project (zooplankton) is still suspended because the project leader has not yet been replaced. One project represents the joint effort of all projects, it synthesizes the results obtained and used the informations obtained from own experiments and literature to build models concerning the kinetics of radioisotopes and stable element in laboratory experiments and in natural ecosystems.
During 1974, the laboratory vessel "Odalisca" was not available because it had to be reclassified and therefore the several aspects of the field programs could not be carried out.

Project No. 1

Title: Physical environmental factors of marine contamination and Special Developments

Name of scientist : M. Bernhard

Results:

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

In order to improve the sensitivity of the apparatus the laminar flow system had to be redesigned. At the same time a red-sensitive photomultiplier was adapted to the system, the '100 channel analyser' was modified so that it would accept impulses of no longer duration and the impulse amplifier modified. The now practically new system was then tested with different size algae especially of small sizes (diameter: 7 μ). This program was carried out in collaboration with Dr. Cervellati from the Laboratorio di Elettronica, CSN - Casaccia.

2) Simulation and model building

A computer program was written in BASIC for the simulation of non-steady-state compartment models. It can be utilized for 2 tracer models (e.g. radioactive and stable element) with up to 25 compartments.

Data generated with the simulation program were used to test the efficiency of different techniques which may be used for the estimation of transport rates from experimental radiotracer data in steady-state. Three different techniques employed were (i) the so-called peeling method or stripping method used to estimate exponentials and coefficients in simultaneous exponential differential equations, (ii) the general method based on the solution of simultaneous differential equations (Sheppard's method) and (iii) a computer program (SAAM 25) which is designed to fit data to a desired model by ad-

justing the parameter values of the model within prefixed limits until a 'best' fit is obtained. Utilizing data with and without a random error from a closed three compartment simulation it could be shown that the transport rates calculated with the aid of the SAAM 25 computer program had the smallest deviations for the theoretical values.
The estimation of transport rates of simulated experiments not in steady-state was also investigated. So far the techniques available allow good estimations only if the systems possess few compartments and if the compartments do not change considerably during the experiments.
These experiences were included summarily in the supporting paper "Use of compartmental models in radioecological laboratory studies" (M. Bernhard, A. Bruschi and F. Möller) prepared for the IAEA Panel "Design of Marine Radioecological Experiments" during its revision.

3) Instrumentation and Apparatus needed by other groups

A programmer is under construction which will be used to partically automatize the different manipulations needed in polarographic determinations of elements in sea water.

4) Collaboration with project 3

Plutonium uptake experiments by unicellular algae were simulated considering different experimental designs in order to facilitate the planning of actual laboratory experiment.

Project No. 2

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

Name of scientist: A. Piro

Results:

1) Investigation of the interaction of zinc with marine sediments in relation to various physico-chemical states of the metal

Scope of this investigation is to correlate the exchange and adsotption reactions of different forms of zinc to the grain size and mineralogical composition of a sediment. Zinc has been selected because its forms and their equilibrium reactions have been investigated in the past years (see Annual Reports from 1968 to 1972, and Piro et al., 1972). The sediment samples have been collected in the Gulf of Taranto in front of Trisaia, where a CNEN Center for Nuclear Research has planned to discharge some radioactive waste into the sea.

For characterization of the sediment an elutriation method has been adjusted, permitting the separation of the sediment in different fractions of desired grain size. For the mineralogical determinations a collaboration with an external laboratory is needed.

The experiments were carried out as follows. A pyrex column, whose inner surface has been equilibrated with the same sea water used in the experiments in order to eliminate any adsorption effect, is partially filled with sea water containing radioactive zinc in a known chemical state (ionic, complexed, etc.). Then, a known quantity of a suspension of the selected grain size fraction of the sediment is introduced. The column vertically held in a rotating support is stopped and let to rotate on its minor axis. By this movement the water layer around the sediment particles is continuously renewed, making the reactions faster. In the same time the air left in the partially filled column flows from one end of the column to the other, producing a certain kind of washing of the inner surface which prevents the adhesion of the particles to the walls.

Only preliminary experiments have been carried out up to now, using a clay fraction of a sample and zinc introduced in the ionic and EDTA-

compl exed forms. Their results are summarized in Fig. 2.1, where the percentage of activity on the particles and in the water are plotted as functi on of the contant time. Zinc in the EDTA-complex form seems to be adsorbed easierly on the particles than that in the ionic form, but further experiments are needed before giving any explanation. These results are intended only as an indication that the used methodology is correct.

2) Automated colorimetric method for the total determination of nitrogen in sea water, culture medium and algae

An automated method for total notrogen determination using the Technicon Autoanalyzer system has been developed. Sea water samples are first oxidized by a $K_2S_2O_8$ solution in a thermostatic bath under cont rolled temperature and pressure (135°C and 3.5 bars). Then the solution in which nitrogen is in the nitrate form is let to react with ascorbic acid in order to eliminate the other undesired oxidation products, mainly chlorine. After this reaction, the solution is automatically analysed for its nitrate and nitrite content by our standard method (Bernhard and Piro, in press). If determinations of total nitrogen in algae are required, a pretreatment of the samples is required. This is accomplished pushing the aqueous algae suspension, mixed with a persulphate solution, through a thermostatic bath at 90°C with a residence time of about 15 minutes, in order to avoid the adhesion of the organisms to the plastic or glass tubes of the manifold. A paper on this method is in preparation.

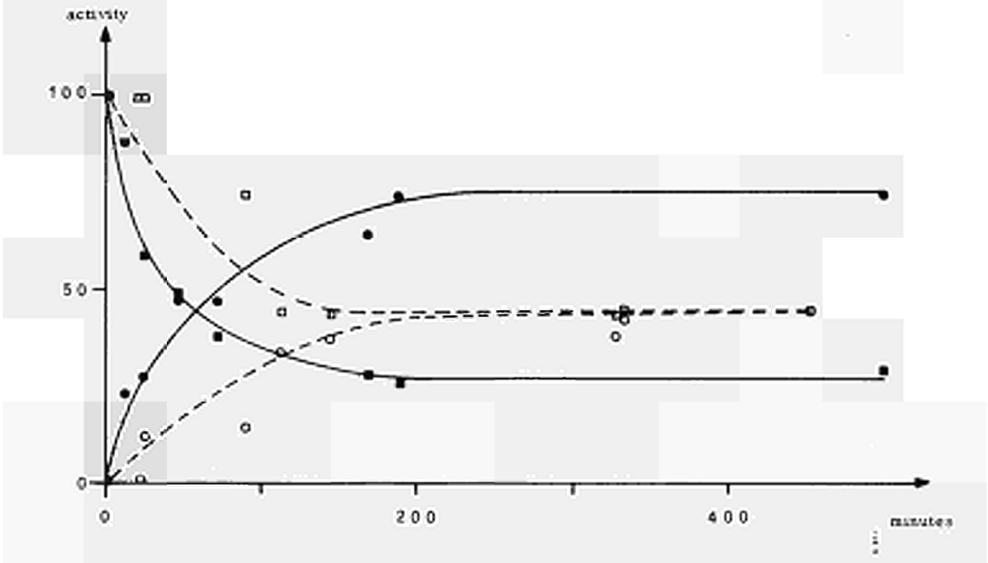

Fig. 2.1 - Percentage of radioactivity in the sediment and in the water as function of the contact time, and the chemical form of radioactive zinc (circles indicate particles; squares indicate water. $^{65}Zn^{2+}$ is indicated by white signs and dashed line; ^{65}ZnEDTA is indicated by black signs and continuous lines).

Project No. 3

Title: The role of phytoplankton in the accumulation, loss and tranfer of radioisotopes in the marine environment

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

Results:

1) Uptake of radioactive caesium

Experiments on the uptake of radioactive Cs have been continued also during 1974. The experiences have been made both with natural and artificial SW. Experiments made with natural sea water media for three species gave at the equilibria (i.e.: when the populations stop to grow and further uptake of Cs does not occur) the following concentration factors: Chaetoceros affinis, 4.4; Phaeodactylum tricornutum, 9.2 and Platymonas suecica, 1.9. In those environments, like estuaries, in which the potassium contents is lower than in normal sea water higher concentration factors for radioactive caesium could be expected, in biota, since caesium can be considered as tracer for potassium. To simulate the above conditions we used artificial sea water with different concentration of potassium and observed that the concentration factors of radioactive Cs can range from 8 in normal artificial sea water to about 35 in artificial sea water in which the potassium contents is reduced to about 5% (~ 27 mg/l) of the content of normal sea water (see Fig. 3.1).

2) Effects of temperature on the growth of phytoplankton

In the near future the nuclear power plants will mostly be placed near to the coastal marine environment. Since the power stations need great quantities of cooling water thermal pollution effects can be expected in the coastal environments. For this reason we

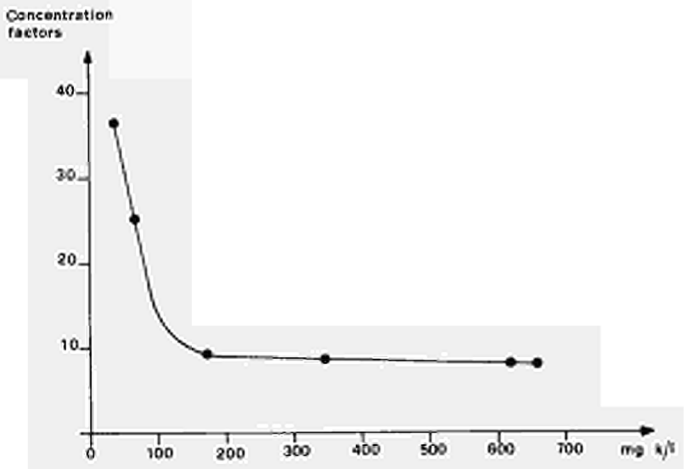

Fig. 3.1 - Concentration factor of ^{134}Cs in Phaeodactylum tricornutum as function of K content in artificial sea water.

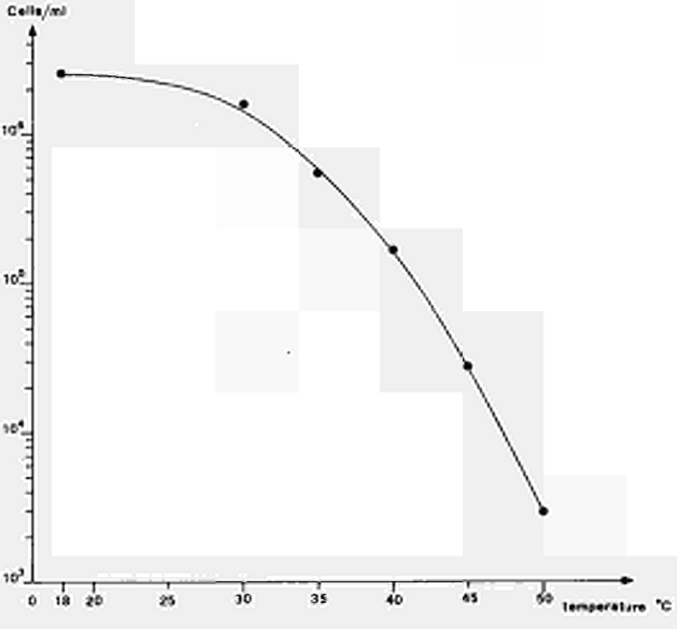

Fig. 3.2 - Survival of Phaeodactylum tricornutum after thermal shock at various temperatures. 18°C is the temperature at which the survival is 100 %. The survival was estimated with the MPN method.

investigated the effects of temperature on the phytoplankton with the aim to get information on thermal schock, long term effects studied in laboratory conditions and effects of temperature detected in a thermal polluted area compared with a non polluted one.

Thermal schocks have been studied with the diatom Phaeodactylum tricornutum which was brought to selected temperature as fast as possible. The scope was to determine the survival with the cultural method (i.e., the MPN method). The results are showed under Fig. 3.2 from which one can see that the lethal temperature for 50% of the cells is 31°C.

In laboratory the long term effects of temperature of 12 phytoplankton strains have been also investigated. The temperatures tested range from 15° to 35°C. The results (see Tab. 3.1) show that up to 25°C no appreciable effect has been observed.

Effects of thermal pollution were studied by evaluating the generation time (during January and Febraury 1974) of Phaeodactylum tricornutum, Platymonas suecica, Asterionella japonica and Glenodinium sp. in a thermal polluted area (near the outfall of the thermoelectrical power station placed in the Gulf of La Spezia) and as comparison in a non polluted one. The temperatures detected in the mentioned areas were respectively 23° and 13°C. The results show no appreciable differences for the two areas. It should be advisable to repeat the experiences when the difference in the temperature of the two areas would be greater (i.e., in summer).

Studies on phytoplankton populations

The data from the in situ experiments carried out for the studies on the effect on the thermal pollution could also be utilized in order to evaluate the growth characteristics (G_T: generation time; and k_T: growth constant) with the scope of evaluating the potential production of phytoplankton in natural conditions similar to those conducted during 1973 (see Annual Report). The prediction of radiocontamination should be made at species level so that the correct identification would have the same accuracy as that of enumeration. For these purposes the construction of a key for the identification of Mediterranean phytoplankton has been continued. A first draft of a key of a good number of phytoflagellatae has been prepared.

Tab. 3.1 - Long term effect of temperature on twelve species of phytoplankton

Algae	Temperatures °C						
	15°C	18°C	25°C	27°C	30°C	33°C	35°C
Prorocentrum micans	0	++++	++++	0	0	0	0
Glenodinium sp_1	+	++++	++++	0	0	0	0
Exuviaella compressa	++	++	+++	0	0	0	0
Coccolithus huxleyi	+++	++++	++++	0	0	0	0
Platymonas suecica	++++	++++	++++	+++	+++	0	0
Phaeodactylum tricornutum	+++	++++	++++	+++	0	0	0
Asterionella japonica	++	++++	++++	0	0	0	0
Chaetoceros danicus	+++	++++	+++	0	0	0	0
Chaetoceros affinis	+++	++++	++++	0	0	0	0
Thalassiosira decipens	++++	++++	++++	0	0	0	0
Skeletonema costatum	++++	++++	++++	++	+++	0	0
Bacteriastrum delicatulum	+++	+++	+	0	0	0	0

++++ normal growth rate
+++ slightly inhibited growth rate
++ half normal
+ no growth, cells alive
0 no growth, cells dead

Project No. 5

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

Name of scientist: E.H. Schulte

Results:

1) Uptake of radioactive zinc by crustaceans Artemia salina

The uptake of ^{65}Zn from sea water in absence of food by brine shrimp nauplii was investigated, because nauplii serve as food for Leander squilla (shrimp) larvae. According to the results nauplii don't reach an equilibrium with the medium during 4-5 days (death after 5 days), because they grew continuously, and therefore the total amount of ^{65}Zn increased continually in the population.

Leander squilla

Accumulation of ^{65}Zn by shrimp larvae from water and from food (Artemia salina) was studied for 4 days (life time of Leander larvae without food: 4-5 days). The results showed that newly hatched Leander larvae accumulated ^{65}Zn rather from food than from water. After 4 days the content of ^{65}Zn in shrimps accumulating ^{65}Zn from water amounted to 10% only of that gained by specimens accumulating ^{65}Zn from food.

Leander squilla larvae, feeding on Artemia nauplii, reached a concentration factor of ~ 300 in radioactive sea water (50 µCi/l ^{65}Zn; specific activity: 18.0), while Artemia concentrated ^{65}Zn up to ~ 2 700 times already after 12 hours exposure. After 48 hours the concentration factor amounted in shrimp larvae to ~1 000 and in Artemia nauplii to ~ 9 000 times. These rapid uptake rates may be due to the very fast metabolism and the high relation between surface to volume of small growing organisms like larvae.

2) Accumulation of ^{65}Zn by shrimp larvae from precontaminated food organisms

In order to eliminate interferences of the amount of ^{65}Zn continuously changing in Artemia nauplii during the experiment caused by growth, the nauplii were precontaminated separately 24 hours before being used as food shrimp larvae.

Leander larvae, living in radioactive sea water (25 µ Ci/l ^{65}Zn, specific activity: 6.5) and feeding on Artemia nauplii, precontaminated for 24 hours in an equal concentration of ^{65}Zn, accumulated ^{65}Zn up to ~ 300 times over water concentration within 24 hours and up to ~ 1 000 times after 48 hours exposure.

In order to maintain the content of radioactivity in the food constant for each day, precontaminated Artemia nauplii were used for 24 hours only and then substituted by new ones.
In the presence of precontaminated food the uptake of ^{65}Zn by Leander squilla larvae approximately tripled, if one takes into consideration the different specific activities used.
During uptake experiments with Leander larvae considerable losses in radioactivity were observed in the larvae. This was most probably caused by the moulting of the specimens. The influence of moults on ^{65}Zn body burden will be studied in further experiments.

3) Influence of radiation of ^{90}Sr on development of shrimp larvae

In concentrations of 10 µCi/l and 100 µCi/l ^{90}Sr neither morphological deformations nor retardation in the larval development (duration of different larval stages) could be observed in Leander larvae; yet ~ 80% of the specimens died in both concentrations tested. In the blank, however, 50% of the larvae survived.

4) Loss of ^{65}Zn by shrimp larvae

During a period of 6 days Leander squilla larvae, living in non-radioactive sea water and feeding on non-radioactive Artemia nauplii, lost equally 50% of the radioactivity (^{65}Zn) whether it was accumulated from water or from food.

Project No. 6

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

Name of scientist: C.N. Peroni

Results:

1) Transfer of radioactivity to copepods and mussels in labelled bacteria in laboratory conditions

The following results have been obtained as far as the transfer of ^{32}P Euterpina acutifrons via labelled bacteria is concerned.

"Sterile copepods", i.e., copepods pretreated in sea water plus 0.1 of penicillin and then incubated in a sea-water medium containing $^{32}PO_4^{---}$ together with labelled bacteria, became radioactive to the same extent as "sterile copepods" incubated without labelled bacteria.

On the contrary, "not sterile copepods", i.e., copepods with their own microflora, incubated in sterile radioactive sea water without labelled bacteria, accumulate 20-30 times more activity than "sterile copepods". This indicated that the bacterial flora associated with the digestive tracts or external surfaces of the copepods, is responsible for the P-uptake. Experiments with copepods subjected to UV radiations for 1 min, seem to indicate that the external microflora is involved in the uptake, unless UV treatment has inhibited the filtration mechanism of the copepods and hence the P-uptake.

If we add UV-inactivated algae to the system (labelled bacteria + copepods in non radioactive sea-water medium) a significant uptake by copepods can be noted which was an order of magnitude higher than the control. In the control i.e., only copepods and bacteria, the P-uptake was negligible.

Similar experiments on transfer of ^{32}P to mussels through contamina-

ted bacteria have been started by incubating four specimens in radioactive sterile sea-water medium in the presence or absence of labelled bacteria. After 2 h and 30 min incubation, the mussels were placed in filtered sea water for three days and the radioactivity was determined. Although the radioactivity in labelled bacteria was only 1% of the radioactivity present as inorganic ^{32}P, the mussels incubated together with contaminated bacteria had a mean activity three times higher than the activity measured in the mussels incubated without bacteria.

This seems to indicate that ^{32}P can be assimulated by mussels through bacteria.

2) Origin or autoradiographic spots from natural marine populations of microorganisms

Experiments dealing with the comparison between autoradiographic spots obtained by bacteria and algae have been carried out in order to study the origin of autoradiographic spots from natural populations. These experiments showed that the flagellate $ß_2$ give spots much greater than those ones produced by the bacterial strain λ.

Project No. 7

Title: Simulation of laboratory experiments and model building of natural and artificial systems

Name of scientists: Joint participation of the other groups

Results:

1) Multi compartment systems

The contributions to this project are mentioned under point 2 of project 1.

2) Survey of a future disposal site (Gulf of Taranto)

In the proposed outfall area of CNEN's Trisaia Center sediments and the most important marine organisms have been sampled for λ and γ analyses.

Publications during the year 1974

BERNHARD, M., 1974 - Studies on the radioactive contamination of the sea. Annual Report, 1972: RT/BIO (74) 1.

BERNHARD, M., A. BRUSCHI and F. MÖLLER, 1974 - Use of compartmental models in radioecological laboratory studies. IAEA Panel Design of Marine Radioecological Experiments. (In press).

BERNHARD, M., M. GHIBAUDO, O. LAVARELLO, C. PERONI and A. ZATTERA, 1974 - A sampler for the aseptic collection of water samples in the sea. Marine Biology, 25, 339-343.

BERNHARD, M., E.D. GOLDBERG and A. PIRO, 1974 - Zinc in seawater: an overview 1975. To be published in: "Nature of sea water" Pergamon Press.

BERNHARD, M., A. PIRO and M. BRANICA, 1974 - Zinc in sea water: distribution of zinc in the Ligurian Sea and Gulf of Taranto (Mediterranean Sea) under special consideration of the sampling procedure. Marine Chemistry (In press).

BERNHARD, M., and A. ZATTERA, 1974 - Radiotracer experiments with benthic algae. IAEA Panel Design of Marine Radioecological Experiments (In Press).

BRANICA, M., M. BERNHARD, and A. PIRO, 1974 - Zinc in sea water: determination of physical chemical states of zinc in sea water. Marine Chemistry (In press).

MÖLLER, F., 1974 - Sistemi di scompartimento trattati come processi stocastici. In: Giornale di Fisica Sanitaria, vol. 18 (1,2) pp. 68-79.

MÖLLER, F., and M. BERNHARD, 1974 - A sequential approach to the counting of plankton organisms. J. Exp. Mar. Biol. and Ecol. vol. 15, pp. 49-68.

MÖLLER. F., and A. ZATTERA, 1974 - The application of sequential estimation methods to counts of phytoplankton. RT/BIO (74) 7 pp. 1-73.

PERONI, C., and O. LAVARELLO, 1974 - La distribuzione dei batteri pelagici nel Mar Ligure ed il loro possibile ruolo nel riciclo dei radionuclidi nell'ambiente marino. Giornale di Fisica Sanitaria e Protezione contro le Radiazioni, Vol. 18 pp. 40-48.

PERONI, C., and O. LAVARELLO, 1974 - Different microbial activities according to depth in the Ligurian Sea as detected by an autoradiographic method. Marine Biology (In press).

PIRO, A., and G. ROSSI, 1974 - Direct automated method for iron determination in sea water. Marine Chemistry (In press).

ZATTERA, A., M. BERNHARD, and C. GALLI, 1974 - Radiotracer experiments with benthic algae. IAEA Panel Design of Marine Radioecological Experiments. (In press).

SCHULTE, E.H., 1974 - Influence of algal concentrations and temperature on the filtration rate of Mytilus edulis. Marine Biology. (In press).

Management Committee

President: Prof. P. KORRINGA
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Collaboration and participation in scientific meetings

As in the past the laboratory has collaborated with the Istituto di Zoologia (Prof. B. Schreiber) Parma, the International Laboratory for Marine Radioactivity (IAEA) Principality of Monaco and the Center of Marine Research (Institute Ruder Boskovic) Rovinj, Yugoslavia.
The 17th Contact Group Meeting on Marine Radioactivity was held at Rovinj, May 5-11, 1974. At this meeting participated besides staff members of the above mentioned laboratories, scientists from Fisheries Radiological Laboratory, Lowestoft, England; the Radiobiological Laboratory of the Biologische Anstalt Helgoland, Hamburg, Germany; the Laboratory, Plymouth, England; Kernforschungsanlage, Jülich, Germany; and C.E.R.B.O.M., Nice, France.
Members of the laboratory took part in the following meetings:
Gruppo di lavoro 5 - Metodi Biologici della Sottocommissione "Inquinamento Acque" (17-18 April, Ispra); IAEA Panel "Effects of ionizing radiations on aquatic organisms and ecosystems" (April 22-26, Vienna); International Symposium "Chemistry of the Mediterranean" (6-8 May, Rovinj); Organizing meeting for the workshop on "Speciation of chemical elements in sea water" sponsored by Dahlem Konferenzen (28-29 July, Berlin); IOC/GFCM/ICSEM-International workshop on "Marine pollution in the Mediterranean" (9 - 14 September, Montecarlo); GFCM Working Party on "Marine pollution in relation to the protection of living resources" (16-18 September, Montecarlo); IAEA Panel "Design of Marine

Radioecological Experiments" (1-5 October, Vienna); Primo Congresso della Associazione Italiana di Oceanografia e Limnologia (5-7 November, Bologna); Euratom-Workshop on "Multiannual programs in marine radioactivity" (14-15 November, Bruxelles); Radioactive and Chemical Committee of CIESM (6-14 December, Montecarlo); Workshop on "Coastal pollution of the Mediterranean" (16-19 December, Copenhagen).

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

Nummer van het contract: 094/72/1 BIAN

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

Algemeen onderwerp van het contract:

RADIATION PROTECTION

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

Algemene omschrijving van de uitgevoerde werkzaamheden:

Main topics of the 1974-research by the soils and plant groups of the Institute were:

- continuation of sampling and analysis in view of the experimental control of the mathematical model concerning the behaviour of ^{90}Sr and ^{137}Cs in soils of Western Europe.
- transport and behaviour of ^{51}Cr, stable Cr, ^{65}Zn, stable Zn, ^{115m}Cd, stable Cd, Hg and mercury compounds in soils. Working out of simulation models for the observed processes.
- kinetics of the uptake and subsequent behaviour of ^{115m}Cd and stable Cd in intact plants and isolated chloroplasts

The work on heavy metals in soils and plants is part of a research programme on "heavy metals in the food chain and in the biosphere" in collaboration with the Biology Group at Ispra-CCR (Italy) and with some other Institutes in Europe.

Research topics on radiation effects in 1974 were:

- further control and extension of a molecular theory on radiation effects in plants and related material, by analysis of literature data and by experimental verification.
- development and improvement of dosimeters and their application in biological research.

The programme for 1974 has once more been carried out in close cooperation with other scientific institutes and organizations. Examples of this scientific collaboration are:

- on different aspects of the application programme within working-groups of the European Society of Nuclear methods in Agriculture (ESNA);
- on pollution, radioactive and other, with the Biology group at Ispra and institutes in the Netherlands, Belgium and Germany;
- on radiation effects within the European working group for Micro-dosimetry;
- on standardization of absorbed dose and dose distribution measurements within the European Late Effects Project Group (EULEP);
- on mutation breeding (vegetatively propagated crops, protein improvement, disease resistance) and incompatibility in higher plants in the Mutation Breeding Contact Group;
- cooperation to projects concerning the testing of irradiated food, wholesomeness testing set up by the Organization for Economic Cooperation and Development (OECD) and the International Atomic Energy Agency (IAEA).
  In this respect also collaboration exists with institutes in the Netherlands, Denmark, Belgium;
- research on genetic control of insect pests, coordinated in Section VII of the TNO working group "integrated control of Insect Pests" and in the joint European Working Group of the "Organisation Internationale de la lutte biologique" (OILB). Cooperation within projects of the IAEA and of the entomology programme of the Biology Division.

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

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Changes in the Scientific Staff

Ir. D. Snieder and ir. S.C.E. Romkes have left the Institute to accept teaching and research duties elsewhere.

New members of the scientific staff are:
ir. W.W.A. Bergers, ir. H. Breteler, dr. F.M. Engels, Miss dr. C.H. Hänisch ten Cate, ir. H. Siebering and ir. P.W.F. de Vrijer, all from The Netherlands.
Temporary members (post-graduate fellows) responsible for particular aspects of the programme: mrs. ir. H.M.G. Ebbens-Groot, ir. F. van Dorp, mrs. M. van Duyvendijk-Matteoli, dr. P. Mix and ir. C. Petit.
Several guest-workers have spent 6 to 12 months at the Institute.

Resultaten van het project No. 1

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra.

Titel van het project:

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

Beschrijving van de resultaten:

The fields concerned have again been sampled in 1974 to a depth of 25 cm.
From the total of 35 samples, 16 have been analyzed and will be counted soon.
The analytical data, collected over the past years will probably be sufficient in number, at the end of 1975, for comparison with forecasts according to the simulation model.

Resultaten van het project No. 3

Hoofd van het team en wetenschappelijke medewerkers:
M.J. Frissel, P. Poelstra, D. Mulder.

Titel van het project:

Behaviour of chromium and other heavy metals in soils

Beschrijving van de resultaten (maximaal 2 bladzijden per project).

Survey of chromium, zinc, and cadmium content of some soils

Five different soils have been selected and analysed for Cr, Zn and Cd by atomic absorption spectrometry, up to a depth of 100 cm.

Some data:

1. Hannover	agricultural area,	permanent pasture, pH (H_2O) : 6.0	
0 - 30 cm	slightly loamy sand	clay 5% org. matter 6%, non calcareous	
30 -100 cm		id. org. matter 2%, non calcareous	
2. Schoonebeek	agricultural area,	permanent pasture, pH (H_2O) : 5.1	
0 -100 cm	peat soil	org. matter > 95%	
3. Alkmaar	agricultural area,	permanent pasture, pH (H_2O) : 6.9	
0 - 30 cm	heavy 'zavel'	clay 20% org. matter 8%, calcareous	
30 -100 cm		id. org. matter 1%, calcareous	
4. Valburg	agricultural area,	permanent pasture, pH (H_2O) : 7.4	
0 - 30 cm	heavy 'zavel',	clay 23% org. matter 6%, calcareous	
30 -100 cm	foreland river Rhine	clay 20% org. matter 1%, calcareous	
5. Biesbos	wild area,	reed culture, pH (H_2O) : 6.9	
0 - 30 cm	heavy clay, fore-	clay 40% org. matter 8%, calcareous	
30 -100 cm	land Rhine delta	id. org. matter 2%, calcareous	

The results are shown in fig. 1. The high concentration in the top layer compared to the concentration of the lower layers is most remarkable. Limiting ourselves to the first three soils, on a first examination of the data, the conclusion that the organic matter of the top layer accumulates the heavy metals seems unavoidable. The observation that the accumulation in the top layer of the peat soil is still more pronounced is not contradictory to this idea. However, it forces one to assume that the contamination must stem from outside (fertilizers, manure, fall out).

A continuous redistribution by worms (a kind of biological extraction), followed by accumulation in the organic material of the top layer, is another explanation. It is, however, generally accepted that worms are responsible for the redistribution of organic material in soil so that this explanation seems incorrect. The same reasoning can be set up for plant roots; as Cr and Hg are almost not transported within the plant roots, this explanation also seems to be incorrect. (See data Hg annual report 1973.) A slow slipping contamination of the top layer of the soil with heavy metals seems probable; investigations to this point will be continued.
The contamination of the forelands of the river Rhine is very high; most remarkable is the absence of a severe Cd accumulation in the Biesbos (fifth row). The other soils confirm more or less the rule-of-thumb that the Zn/Cd ratio often equals 100:1. For the Biesbos this figure is 1000:1. Both less deposition and better mobility may explain this observation.
Literature reports that Cd is less adsorbed than Zn; we could not confirm this fact. Typical adsorption data for Cd are presented in fig. 2. The ratio Cd (adsorbed)/Cd (solution) ranges from 39 ml g^{-1} for the 30-40 can layer of the sewage field at Braunschweig to 703 ml g^{-1} for the Valberg clay. Such unique values can not be given for Zn. Contradictory to Cd, even at levels of 0.002 meq Zn per ml solution, the adsorption isotherm is curvilinear. Furthermore the adsorption characteristics depend on the adsorption time. Because of this last point, the measurements are not yet rounded off. Values rage from approx. 10 ml g^{-1} for the lower layers of the sewage field at Braunschweig, to approx. 1000 ml g^{-1} for the Valberg clay.
The experiments on undisturbed soil columns are less 'sensitive' because of the slow rate at which adsorption equilibrium occurs. The column experiments are still in progress; a first conclusion can be that Cd is not leached out faster than Zn.
Another feature, studied in the columns, is the influence of anaerobic conditions in the soil. There is not much reason to assume that the Zn and Cd ions themselves will change as a result of a low redox-potential, but many other ions will change indeed. Among these, Fe and Mn ions compete with Zn and Cd ions for places on the adsorption complex, and, as a result, the adsorption curves of Zn and Cd may change. Till now we noticed for Zn under anaerobic conditions a higher migration rate than under aerobic conditions.

This phenomenon may be of importance for the contamination of ground water under refuse dumps. Samples taken from a subsoil of a refuse dump with controlled tipping (sanitary landfilling) at Delden were analysed for heavy metals in cooperation with Stichting Verwijdering Afvalstoffen, Amersfoort and Institute for Soil Survey, Wageningen). Highest amounts for the 0-5 cm layer were: Fe 10900 ppm, Zr 220 ppm, Zn 82 ppm and Ce 34 ppm. At a depth of 75 cm the levels for those ions were Fe 2900 ppm, Zn 107 ppm, Zn 27 ppm and Ce 19 ppm. From these differences and the fact that the Zn level of a subsoil of a non contaminated field equals 10 ppm, it can be concluded that contamination takes place indeed. Table 1 provides details of the determinated data.

Table 1

Concentration of heavy metals in the subsoil of the refuse dump at Delden (n.d. = not determined).

Depth cm	Texture, colour	Element								
		Sb	Eu	Co	Fe	Ta	Tb	Sc	Rb	Cs
0- 5	humic loamy fine sand	0.9	0.4	2.1	10934	0.5	0.1	0.7	21	1.3
5-15	" "	n.d.	0.3	1,8	9150	0.4	0.1	0.6	21	1.1
15-25	" "	0.6	0.2	0.9	5156	0.5	0.1	0.4	n.d.	0.7
25-35	" "	0.4	0.2	0.7	4238	0.4	0.1	0.4	17	0.7
35-45	" "	0.5	0.2	0.9	5339	0.4	0.1	0.4	20	0.9
45-55	loamy fine sand, green shade	0.4	0.2	0.8	2878	0.3	0.1	0.3	19	0.8
55-65	" "	0.5	0.2	0.9	3031	0.3	0.1	0.4	20	0.8
65-75	" "	0.4	0.2	1.1	2900	0.3	0.1	0.3	20	0.8

Depth cm	Texture, colour	Element								
		Hg	Zr	Hf	Cr	Yb	Se	Ce	Zn	Sr
0- 5	humic loamy fine sand	0.12	219	8.3	41	0.3	1.7	34	82	n.d.
5-15	" "	0.11	209	7.8	37	0.3	1.4	29	60	n.d.
15-25	" "	0.03	185	7.3	22	0.2	1.3	22	29	n.d.
25-35	" "	0.03	200	7.7	22	0.2	1.2	17	32	n.d.
35-45	" "	n.d.	145	5.9	18	0.1	1.3	16	26	n.d.
45-55	loamy fine sand, green shade	n.d.	95	3.8	20	0.1	0.9	15	20	n.d.
55-65	" "	n.d.	153	5.8	21	0.1	0.8	17	29	n.d.
65-75	" "	n.d.	107	4.1	17	0.1	0.5	19	27	24

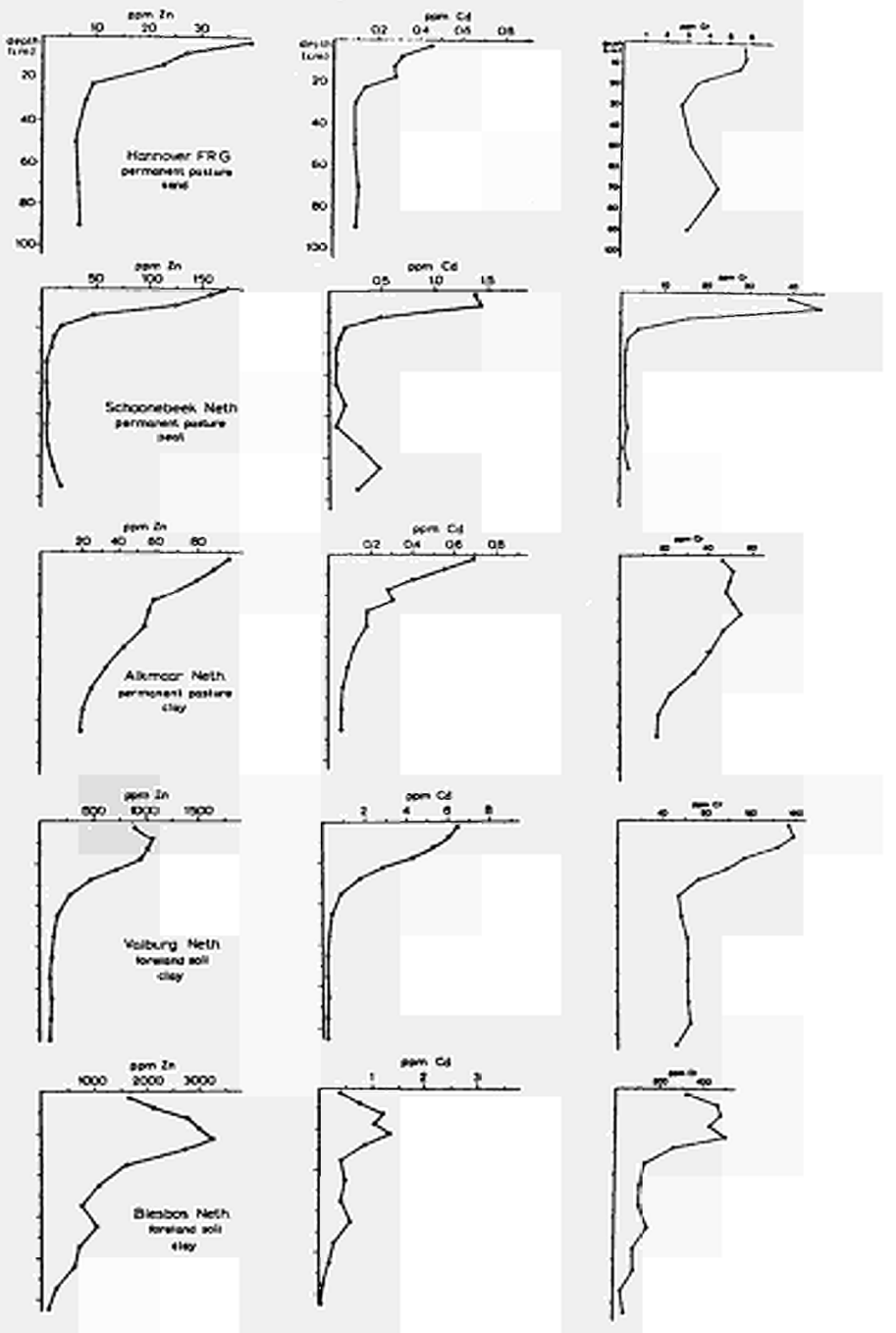


Fig. 1 The distribution of total Zn, Cd and Cr in a number of soil profiles. Note the differences in scale of the horizontal axis.

Publications - 1974

El-Brassom, N., Poelstra, P. and Frissel, M.J.: Chrom und Quecksilber in einem seit 80 Jahren mit städtischen Abwasser berieselten Boden.
Accepted for publication by Z. Pflanz. u. Bodenk.

Reiniger, P., Poelstra, P. and Frissel, M.J.: Chromium in soils.
Submitted for publication.

Other publications on heavy metals are listed in annual report project 4 of this programme (mercury).

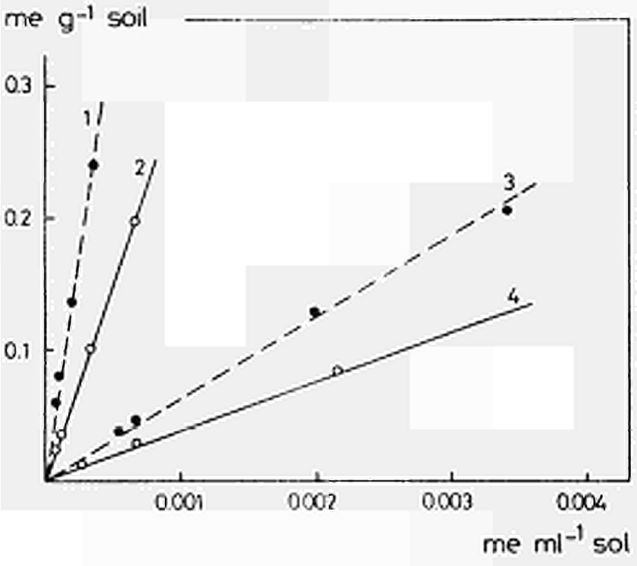


Fig. 2 Isotherms for the adsorption of Cd ions on soil.
1. Valburg, Cd (ads)/Cd (sol) = 703 ml g^{-1}; 2. Schoonebeek, Cd (ads)/Cd (sol) = 322 ml g^{-1}; 3. Braunschweig 0-20 cm, Cd (ads)/Cd (sol) = 64 ml g^{-1}; 4. Braunschweig 30-40 cm, Cd (ads)/Cd (sol) = 39 ml g^{-1}.

Resultaten van het project No. 4

Hoofd van het team en wetenschappelijke medewerkers:

Frissel, M.J. and P. Poelstra

Titel van het project:

Behaviour of mercury and mercury compounds in soils

Beschrijving van de resultaten:

At its final stage this report contains the main conclusions of the investigations carried out over the past years:

- The mercury compounds $HgCl_2$, Hg Cl CH_3 and metallic mercury are very immobile in soil as far as physico-chemical processes are concerned.
- The volatile mercury compound $Hg(CH_3)_2$ is very mobile in soil via the gas phase.
- The conversion of mercury compounds into organic volatile compounds, like methylchloride is a very slow process in soils.
- All organic mercury products, $Hg(CH_3)_2$ included, are converted into inorganic mercury in soil. This inorganic mercury is strongly adsorbed on the soil. Identification of the adsorbed mercury compound was not possible.
- Typical mercury data for soils are:

Forelands River Rhine	1 - 10 ppm
Bulb soils (0 - 50 cm depth)	0.15 ppm
Pastures, industrial areas (0 - 20 cm)	0.10 ppm
Pastures, non industrial areas (0 - 20 cm)	0.04 ppm
Pastures, subsoils (80 - 100 cm)	0.01 ppm

- The contamination of areas which were never flooded with polluted water due to mercury containing chemicals, can be estimated due to

$$Hg_{drain,t} = a.R.\ Hg_{air,t} + b.\ Hg_{degas}$$

in which:

- $Hg_{drain,t}$ = mercury drain rate as f(time)
- a = conversion factor (tons produced → ppm)
- R = factor which accounts for an unequal mercury distribution on the surface of the globe. For industrial areas in the Netherlands, Germany and Italy R = 10; for more quiet areas R = 5; for the south of Italy R = 1 (which indicates that no extra contamination occurs).
- $Hg_{air,t}$ = man produced mercury released into the atmosphere as f(time)
- b = conversion factor (tons produced → ppm)
- Hg_{degas} = annual natural mercury release into the atmosphere.

$Hg_{air,t}$ is calculated by:

$$Hg_{air,t} = C_t \times CFR + O_t \times OFR + 0.03 \times P_t + 0.43 \times 0.97 \times P_t \ (1-RCF)$$

in which: C_t = coal production as f (time)
O_t = oil production as f (time)
P_t = Hg production as f (time)
CFR = Hg conc. in coal
OFR = Hg conc. in oil
RCF = Hg recycling fraction

-Typical data for the mercury balance in the Netherlands:

mercury consumption	100	t/year
mercury in Rhine (input)	70	t/year
mercury in Rhine (output)	15	t/year (estimate)
'agricultural' applied mercury	2	t/year (still decreasing)
contribution from atmosphere	9	t/year (speculative calculation)

More details can be found in the earlier annual reports of the association and the papers by Poelstra et al. (1973) and Frissel et al. (1974). Results were discussed at the IAEA/FAO meeting at Helsinki 'Comparitive aspects of food and environmental accumulation' and local meetings (CNB - TNO working group for mercury, TNO working group 'Transport and Accumulation Phenomena related to Soil Contamination', Commission for Phytopharmacy, subgroup contamination of drinking water).
Concluded was that the failure to produce $Hg(CH_3)_2$ in the laboratory does not exclude $Hg(CH_3)_2$ production in nature. Because also van Faasen, (Institute for Soil Fertility, Haren) and other authors reported negative results, it was decided not to start again the study on the methylation of mercury which was discontinued after the departure of van der Steene, nov.1973. It was Beckert, Las Vegas (Nat. june 1974) who succeeded to prove both, in the laboratory and on fields of the Nevada test side, that transition of Hg into organic compounds is possible. His report is too brief to consider differences in ecological parameters.
The calculation of the mercury drain rate is very speculative; only if pertinent data on the mercury drain become available a further evaluation of the data seems justified. The present determinations of mercury in the air are concentrated on the mercury content in the air itself, not on the rain out of mercury. A survey of changes in the mercury content in the soils studied, to be carried out every 2 years, was suggested. The vegetation of the foreland soils of the river Rhine will be included in this survey. During this project nine publications were prepared, the latter three of them appeared in 1974 and are listed in appendix 1c. With this evaluation the project is terminated. Remaining studies on mercury fit well within the scope of the radiaton protection project n° 3 on the behaviour of heavy metals.

Publications - 1974

POELSTRA, P., M.J. Frissel, N. van der Klugt and W. Tap, Behaviour of mercury compounds in soils: accumulation and evaporation. Comparative aspects of food and environmental accumulation. IAEA, Vienna, 1974 p.281-291.

FRISSEL, M.J., P. Poelstra and N. van der Klugt, The contamination of Dutch soils with mercury and a few other heavy metals. Geologie en Mijnbouw 53, 163-170 (1974).

FRISSEL, M.J., and P. Reiniger. 'Transport of micro-amounts of strongly adsorbed compounds in the field' in Simulation of accumulation and leacking in soils, Pudoc, Wageningen 1974, p. 31-38.

Resultaten van het project No. 5

Hoofd van het team en wetenschappelijke medewerkers:

G. Verfaillie, C. Petit.

Titel van het project:

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

Beschrijving van de resultaten:

The kinetics of cadmium uptake by 6 weeks old tomato plants (*Lycopersicon esculentum*, Mill, cv. Moneymaker) has been studied using the set-up and the methods described in the previous annual reports of the project. All kinetic runs described below have been realized with batches of 3 plants and with ^{115m}Cd as tracer of the Cadmium. Four parameters have been taken into account, namely: contamination schedule, ionic strength, Cd^{++} concentration and temperature of the nutrient solution.

1. The influence of the contamination scheme (time and frequency).

The cadmium uptake efficiency being markedly high, a run leading to complete exhaustion of the cadmium from the nutrient solution is performed within a few hours. Therefore, several replicates of labelled cadmium injections could be done without changing the batch of experimental plants. Doing this, it has been observed that the initial rate of cadmium uptake, for repeated runs at constant initial 10^{-7}M Cd^{++} concentration, strongly increased from run to run with the time elapsed since the beginning of the first contamination, tending towards a limiting value after about 2 days or 6 completed runs (fig. 1a). When an initial Cd^{++} concentration of 10^{-6}M was used, a similar effect was observed but the uptake activation proceeded more quickly towards the limiting value. This suggests that the "time effect" might be due to a metabolic self activation of the cadmium uptake process. This hypothesis was confirmed by the apparent suppression of the activation when the successive complete runs were replaced by short initial phases of similar runs with renewing of the solution between each of them. Proceeding in this way, indeed, the total amount of cadmium that accumulates in the plants during an experiment is severely reduced. Consequently the concept of "time effect" might better be replaced by that of "contamination effect" and the variation affecting the uptake rate by expressed in function of the total amount of cadmium accumulated in the plants as in fig. 1b. According to this mode of representation, the uptake rate would increase linearly with the accumulated amount of cadmium until a saturating value of the latter would be reached. Such an interpretation would also confirm the hypothesis of metabolic self activation.

2. The influence of the ionic strength.

The plants respond immediately to an increase of ionic strength of the nutrient solution, containing 10^{-7}M Cd^{++}, by a decrease of the cadmium uptake rate as it is shown in fig. 2. This effect can easily be explained by the

lowering of the negative electric charge of the root surface accompanying any increase of the ionic strength of the nutrient solution. This lowering, indeed, must result in a reduced attraction of the Cd^{++} cations.

3. The influence of the Cd^{++} concentration.

The influence of the Cd^{++} concentration on the rate of cadmium uptake has been followed using the step wise scanning method already described in 1973 for the uptake of chromium. The kinetics of the Cd^{++} uptake in the concentration range 10^{-7}M to 10^{-4}M presents at least two absorption isotherms represented by the solid lines in fig. 3 on which the corresponding kinetic parameters, expressed in terms of Michaelis hyperbolic formulation, are also given. Below the threshold concentration, the hyperbolic function rather fairly fits the experimental points obtained for "Mechanism I". The fitting of the second isotherm corresponding to "Mechanism II" is much more questionable and the existence of manifold components might be suggested and represented by the dashed lines in fig. 3. As in the case of chromium uptake, the threshold concentration corresponds approximately to the toxicity level for cadmium.

4. The influence of the temperature.

The effect of the temperature on the cadmium uptake from a 10^{-7}M Cd^{++} nutrient solution has been measured for temperatures ranging from 5°C to 50°C. The uptake rate rises with the temperature up to a maximum between 30°C and 35°C, then decreases more and more rapidly and is almost zero at approx. 50°C. If the temperature is kept at this last level, the plants begin to release cadmium into the solution after a delay of about 2 hours. The behaviour of the cadmium uptake toward temperature variations as it is represented in fig. 4a is quite similar to that of an enzymatic activity. Applying the Arrhenius equation which is valid for enzymes before they undergo thermic denaturation, we have:

$$V = A\, e^{-E/RT} \qquad \text{or} \qquad \ln V = \ln A - E/RT$$

where V is the cadmium uptake rate, A a constant, E the activation energy, R the gas constant and T the absolute temperature.
Plotting the logarithm of the cadmium uptake rate against the inverse of the absolute temperature gives in fig. 4b an excellent fitting of the expected linear relation with a correlation coefficient as high as 0.998. From the slope of the straight line, the value of the activation energy E is readily computed and found to be 15 Kcal per mole of cadmium. Such a relatively high activation energy implies that the cadmium uptake is dependent on the general metabolism of the plants.

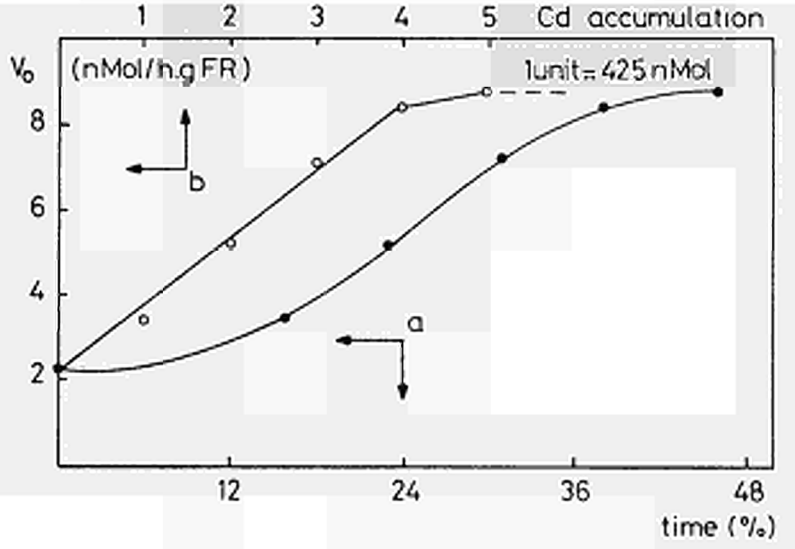


Fig. 1 - Effects of time and/or cadmium accumulation in plants on the initial rate of cadmium uptake.

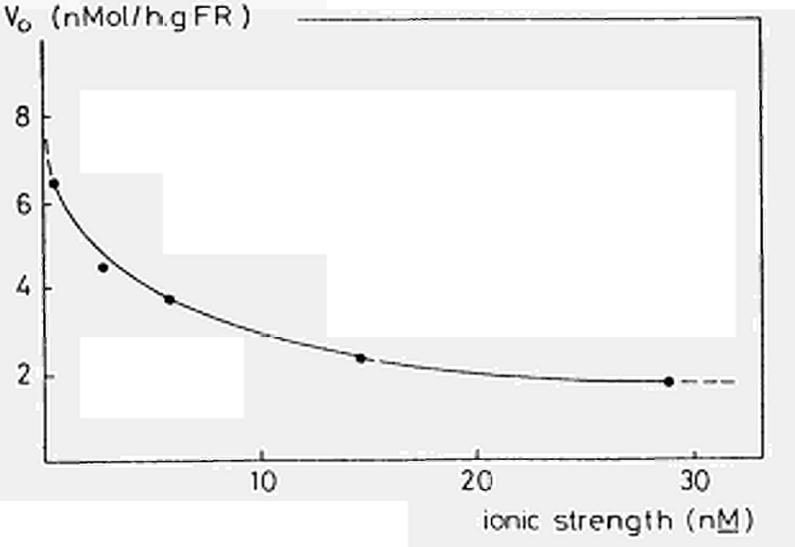


Fig. 2 - Effect of the ionic strength of the nutrient solution on the initial rate of cadmium uptake.

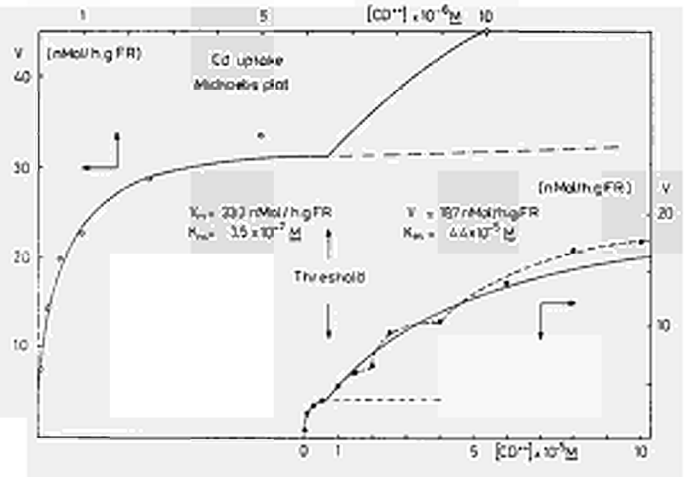
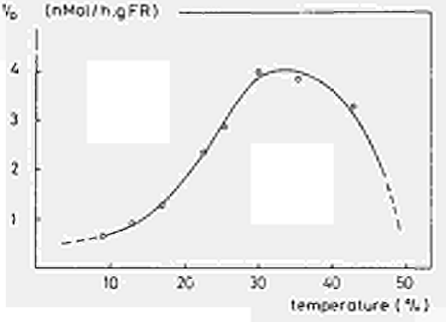


Fig. 3 - Effect of Cd^{++} concentration on the initial rate of cadmium uptake. Michaelis plot.



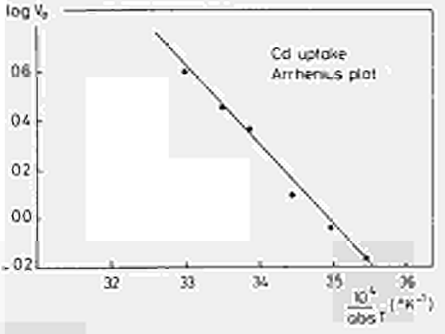


Fig. 4 - a. Effect of temperature on the initial rate of cadmium uptake.

b. Linearization of the Arrhenius plot.

Publications - 1974.

VERFAILLIE, G.R.M. Kinetics of chromium absorption by intact rice plants.
Proceedings of the symposium on "Comparative Studies of Food and Environmental Contamination" 1974, IAEA-SM-175, 315-331.

Biology Division, C.E.C., collaboration between Biology Group, Ispra and Association EURATOM-ITAL, Wageningen.
The behaviour of chromium in aquatic and terrestrial food chains. (in press).

Resultaten van het project No. 6

Hoofd van het team en wetenschappelijke medewerkers:

C. Petit, G. Verfaillie.

Titel van het project:

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

Beschrijving van de resultaten:

Using ^{115m}Cd as tracer, the behaviour of cadmium absorbed by the roots of intact tomato plants (*Lycopersicon esculentum* Mill, cv. Moneymaker) has been followed by means of three different tracing techniques.
The kinetics of the absorption itself has been studied by continuous recording of the ^{115m}Cd exhaustion from the nutrient solution. The results concerning this part of the study are detailed in the report of project 5 of the radiation protection programme.
The transport of cadmium in the stem has been followed *in vivo* with semi-conductor detectors. Transversal movement of the cadmium from the xylem has been detected by beta ray spectrometry, according to a method described in the report of project 30 of the applications programme.
The longitudinal movements have been determined by comparing the simultaneous responses of several detectors applied to various parts of the plants. The cadmium moves from the stem to the petioles and then to the margin of the leaves.
To verify the results concerning the transport of the cadmium, the distribution of the latter has been determined by autoradiography of similar whole plants after various periods of cadmium accumulation. All plants used were grown on Hoagland-Arnon I nutrient solution for one month, including a final period of contamination by labelled cadmium lasting according to the treatments from 1 hour up to 15 days. During the contamination period, the concentration of the cadmium in the nutrient solution was maintained at 2×10^{-6} M Cd^{++}. During the first two days of its uptake, the cadmium is uniformly distributed in all aerial parts, disregarding the age of the latter (figure 1). After 15 days of uptake, cadmium is present in all parts of the plant but mainly accumulates in roots, limbs and margins of old leaves (figure 2). With respect to this, it must be emphasized that the limbs of old leaves also show an important yellowing when the Cd^{++} concentration of the nutrient solution exceeds 2×10^{-6} M. Some plants having accumulated cadmium during 35 hours were transferred to a cadmium-free Hoagland-Arnon I solution and analysed by autoradiography a week later (figure 3). Although no release of cadmium could be detected in the nutrient solution, the cadmium almost disappeared from the stems, petioles and veines and concentrated in the margins of the leaves and in the roots. From all these results, we conclude that the pathway of the longitudinal cadmium transport in the

aerial parts is the same as that of the transpiratory flux. The observations made *in vivo* with semiconductor detectors are confirmed by the autoradiographies. To visualize the transversal movement, cross-sections of the stem have also been made and are presently submitted to micro-autoradiographic analysis.

Fig. 1 - Autoradiography of a one month old tomato plant after 35 hours of ^{115m}Cd uptake.

Fig. 2 - Autoradiography of a one month old tomato plant after 15 days of ^{115m}Cd uptake.

Fig. 3 - Autoradiography of a one month old tomato plant after 35 hours of ^{115m}Cd uptake followed by a one week growth on a cadmium-free nutrient solution.

Resultaten van het project No. 7

Hoofd van het team en wetenschappelijke medewerkers:

G. Desmet, M. van Duyvendijk-Matteoli, A. de Ruyter.

Titel van het project:

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

Beschrijving van de resultaten:

The action of heavy metals on the physiology of plants cannot be understood without better knowledge about the behaviour of physiological elements such as Mn^{++}, Zn^{++}, Ca^{++} etc, and vice-versa. In connection with this statement some prelimenary data on the behaviour of Mn^{++} are presented in the report of project No 7, of the Applications programme. The action of Cadmium on the metabolism of isolated chloroplasts has been the subject of the present investigation. It was shown that Cd inhibits the electron transport and deteriorates the energy conservation of those organells. A detailed study is in progress in order to establish the exact localization of the action of Cd on the electron transport chain. Hitherto, it was found by oxygraphic measurements that the inhibition of the photosynthetic electron transport, due to the presence of Cd is bypassed by the action of NH_2OH (fig 1). Cadmium thus is an inhibitor of the H_2O splitting enzyme, (a protein containing manganese), since NH_2OH is known to donate electrons to a site (containing also a Mn-protein) precisely behind that protein. These findings may be presented in the following scheme:

$$H_2O \text{ — } \underset{\uparrow Cd}{\text{Mn prot}} \rightarrow \underset{\uparrow NH_2OH}{\text{Mn prot}} \rightarrow D_1\text{chl } a_{II}\ Q \xrightarrow[\text{carriers}]{\text{electron}} \text{chl } a_I \rightarrow \text{electron acceptor}$$

Externally added Mn^{++} ions are in concurrency with H_2O as an electron donor, and decrease the concentration range of Cd necessary to inhibit the chain (fig 2). This concentration range furthermore depends on the amount of ligand (Cl^-) present in the medium (fig 3). This is a good indication for the fact that the amount of Cd present in the chloroplast results from the competition between Cl^- and the chloroplast-for Cd^{++}. In order to make the connection between what happens in isolated organelles and the metabolism of whole plants, the following experiments were done:

Macroautoradiography showed that ^{115m}Cd is uniformly distributed within 36 hours after root application in the entire plant and, therefore, also in the spinach leaves. The toxicity range of cadmium for spinach plants (Spinacea oleracea L var Verbeterd Breedblad), grown on a complete mineral solution, has been determined.

The solution contained the normal concentration of manganese, i.e. 10^{-5} M and toxicity effects were observed in the range from 10^{-6} M upto 8.10^{-5} M cadmium. In order to inhibit the metabolism of chloroplasts much higher concentrations have to be used, i.e. from 10^{-3} to 10^{-2} M.
It should be mentioned, however, that the amount of Cd present in the leaves is considerably higher (approx. 4 times) than the one e.g. Mn. Therefore an accumulation effect may be involved. It was found in fact from the biochemical experiments, mentioned above, that the toxic action of Cd on isolated chloroplasts was affected by its interaction with Mn. The metabolically important Mn level in the spinach plant may be correlated positively with the Cd level, and thus influence the toxicity range of Cd. Final quantitative results about this interaction are not yet available. Nevertheless it can already be mentioned that the Cd-toxicity range of chloroplasts, isolated from 'low but non-deficient manganese' plants, is shifted towards lower Cd concentrations, and thus depends on the amount of manganese present in chloroplasts.

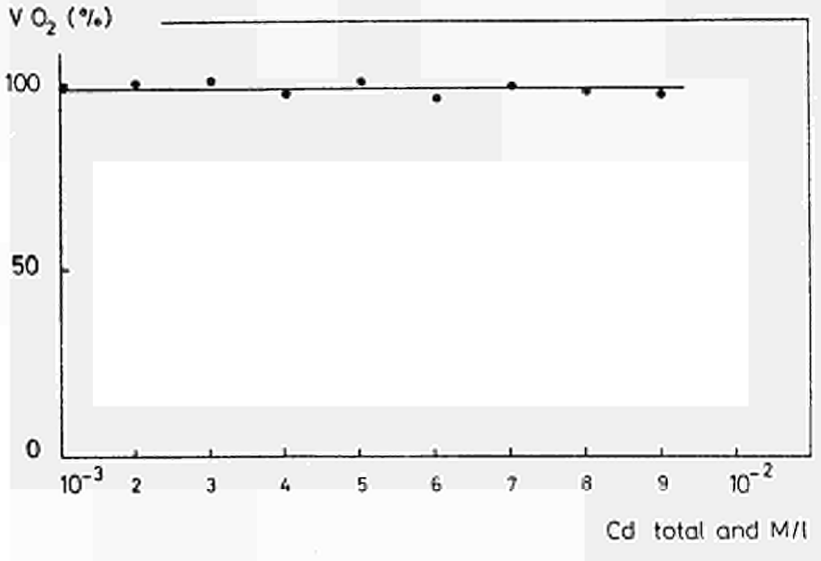


Fig.1. Influence of a Cd^{++} treatment at different concentrations on the rate $(V_{O_2})_2$ of the electron transport in chloroplasts, treated with 5.10^{-2} M NH_2OH.

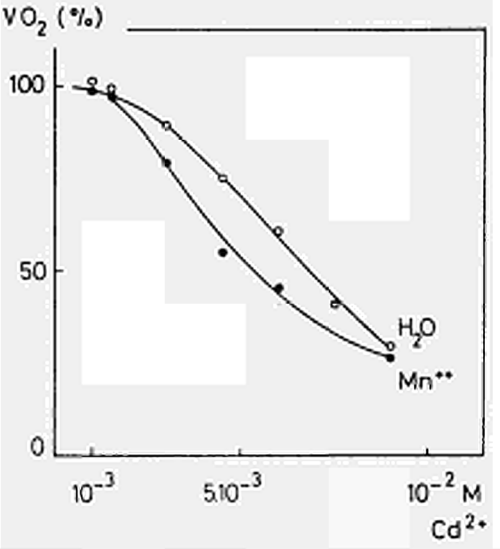


Fig.2. Effect of externally applied Mn^{++} ions (10^{-2}M) on the concentration range of Cd^{++} which is toxic for metabolical active chloroplasts.

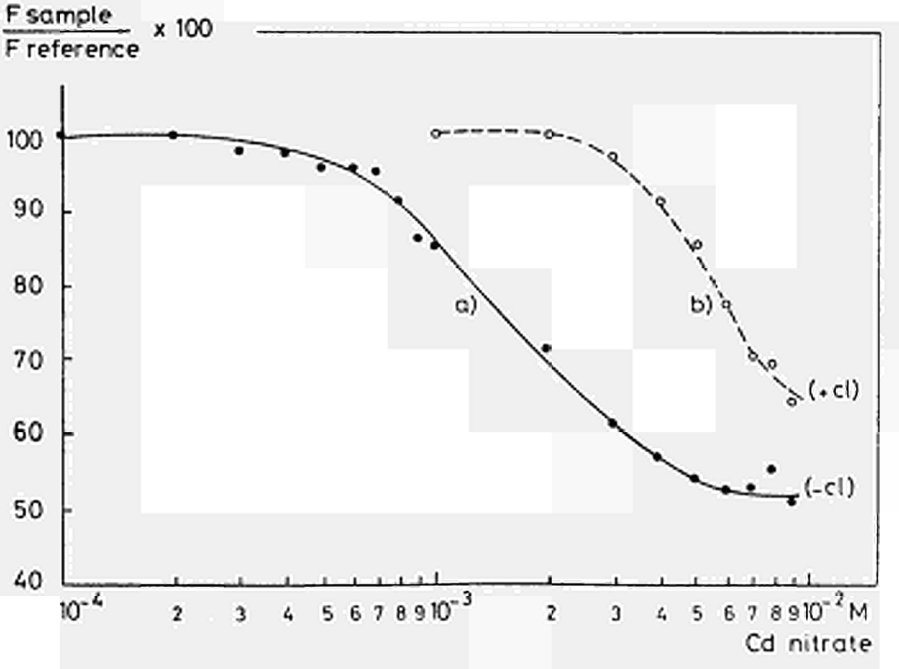


Fig.3. Influence of the ligand (Cl^-) concentration on the inhibition of the Mehler reaction by Cd^{++}.

Publications 1974

DESMET G., DE RUYTER A., RINGOET A.
Absorption and metabolism of CrO_4^{2-} by isolated chloroplasts. Phytochemistry (in press).

M. VAN DUYVENDIJK-MATTEOLI, G. DESMET.
Inhibition and uncoupling of the electron transport in isolated chloroplasts by cadmium (in preparation).

Resultaten van het project No. 8

Hoofd van het team en wetenschappelijke medewerkers:

K.H. Chadwick, H.P. Leenhouts, K.J. Puite, W.F. Oosterheert.

Titel van het project:

Primary radiation effects in inert and biological material.

Beschrijving van de resultaten:

Results concerning the molecular theory:

Post-irradiation effects.

The model predicts that the change in survival, caused by certain post-irradiation storage treatments, should be due to the repair of DNA double strand breaks and that a relationship should exist between survival with and without post-irradiation treatment.

If: $S_1 = e^{-p(\alpha D+\beta D^2)}$ is normal survival then after post-irradiation storage: $S_2 = e^{-f_o p(\alpha D+\beta D^2)}$ where $f_o < 1$ and represents repair. Then

$$\ln S_2 = f_o \ln S_1 \qquad (1)$$

Several published results have been analysed and found to be in accordance with equation (1), Fig. 1, and the experimental circumstances under which post-irradiation repair takes place are compatible with those needed for the repair of DNA double strand breaks. In addition the repair of DNA double strand breaks has recently been shown to occur in eukaryotic cells following radiation.

Mutations vs cell death.

In the development of the molecular theory, an equation has been derived which directly relates the mutation frequency per survivor and the surviving fraction of cells following ionising radiation. This equation can be generalized for other mutagenic treatments. When a mutagenic treatment leads to cell death and to the induction of a mutation, both originating from the same type of critical lesion, then for a uniform population of cells the following relation is given:

$$\ln S = - (p/q) M \qquad (2)$$

where S is the surviving fraction
p, the probability that a lesion leads to cell death
q, the probability that lesion leads to the mutation and
M is the mutation frequency per surviving cell.

This equation is independent of the way, or the kinetics, by which the lesion is formed and can be applied to different mutagenic treatments, such as ionizing radiation, U.V. light, and chemicals.
Data from literature have been used to test the predicted relation and it has been shown that the data of several investigators could

quite well be described by equation (2), (Fig.2) independent of the mutagenic treatment or the dose (concentration-time). It seems possible that this analysis could be used with a specifically selected mutagens to learn more about the basic mechanisms involved in the formation of the initial lesion.

LET effects.

For the calculation the coefficient "a" of the molecular theory equation $S = e^{-aD-bD^2}$ as a function of radiation quality the geometric dimensions of tracks need to be known. Only in the classical approach an estimate is given of the radius of a track: R (:) β for $\frac{z}{\beta} << 137$.

In order to estimate the dimensions of a track for all values of β a calculation of the stopping power was set up based on:

1. the general quantum mechanical scattering cross section

$$/f/^2 = t\,\frac{z^2}{\beta^4}$$

2. the limiting conditions of the Coulomb interaction of the ionizing particle (the Heisenberg relation and the collision time).

3. the average energy transferred in the primary collision calculated from the optical and excitation spectra given by R.L. Platzman.

The stopping power calculated in this way, for water is quite reasonably in accordance with the experimental data, both for ions and electrons, and it turned out that the dimensions of the track are strongly dependent on β over the total range of energies.
The insight gained with this track structure model will be used to calculate the "a" coefficients as a function of radiation quality using the experimental data of Barendsen and Todd on T1 human kidney cells.

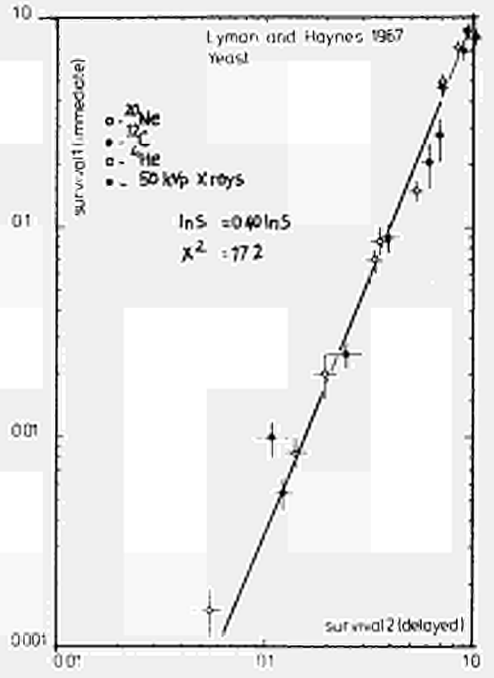


Fig. 1 - Analysis of the post irradiation treatment of yeast cells for different radiations according to the equations $\ln S_2 = f_0 \ln S$.

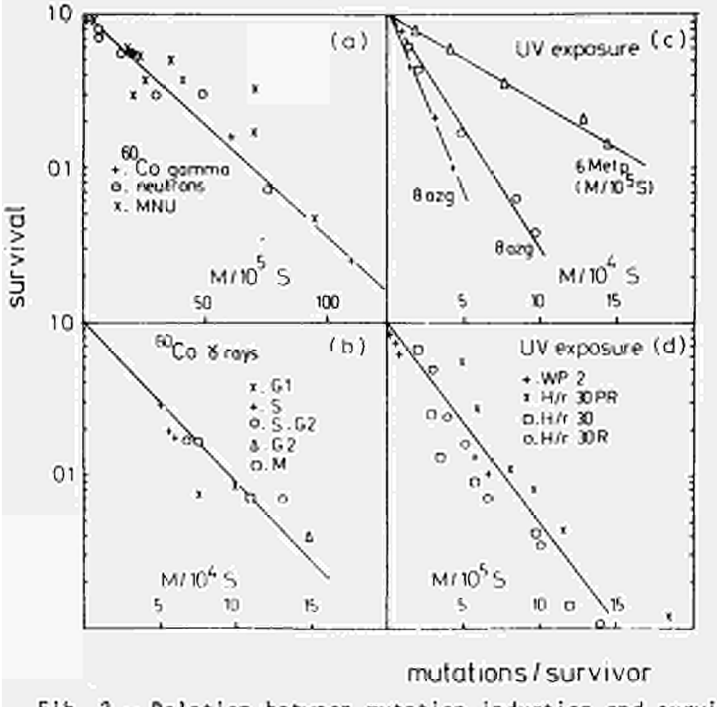


Fig. 2 - Relation between mutation induction and survival following different exposure treatments analysed according to the equaiton $\ln S = -(p/q)M$.

Publications - 1974.

CHADWICK, K.H. and H.P. LEENHOUTS. A common molecular mechanism in radiobiology, its implications in radiological protection.
Proceedings of 3rd IRPA Conference CONF-73097 pp 457-468 (1974).

CHADWICK, K.H. and H.P. LEENHOUTS. Chromosome aberrations and cell death.
Proceedings 4th Microdosimetry Symposium EUR 5122 d-e-f pp 585-606 (1974).

CHADWICK, K.H. and H.P. LEENHOUTS. Repair of potentially lethal damage: an alternative approach.
Radiation and Environmental Biophysics (1974) in press.

LEENHOUTS, H.P. and K.H. CHADWICK. DNA double strand breaks and chromosome aberrations.
Theoretical and Applied Genetics 44, 167-172 (1974).

LEENHOUTS, H.P. and K.H. CHADWICK. A theoretical analysis of radiation sensitivity in cells following neutron irradiation.
Biological Effects of Neutron Irradiation, IAEA Vienna SM-179 pp 151-163 (1974).

LEENHOUTS, H.P. and K.H. CHADWICK. The RBE-LET relationship.
Proceedings 4th Microdosimetry symposium. EUR 5122 d-e-f pp 381-404. (1974).

CHADWICK, K.H. and H.P. LEENHOUTS. The effect of an asynchronous population of cells on the initial slope of dose-effect curves.
Paper presented at 6th Memorial L.H. Gray Symposium London Sept. (1974).

Resultaten van het project no. 9

Hoofd van het team en wetenschappelijke medewerkers:

M.A. Hannan, K.J. Puite, P.A.T.J. Werry.

Titel van het project:

Irradiation dose-mutation relation in rad dose range.

Beschrijving van de resultaten:

A. Experiments with Tradescantia pollen

After exposure of inflorescences of Tradescantia paludosa to various radiation conditions, chromosome aberrations at pollen mitosis and inhibition of the germination capacity of the pollen grains will be scored to see if these end-points are closely correlated as may be expected from the recently developed molecular theory. Preliminary germination experiments using X-rays with exposure rates of 290 R/min, 95 R/min and 24 R/min have been performed. Changes in the shape of the 'survival' curves due to a change in exposure rate are observed.
Experience has been obtained with the cytological techniques for scoring the radiation induced chromosome aberrations.

B. Experiments with the fission yeast Schizosaccharomyces pombe

To collect data on cell survival and mutation frequency in both G1 and G2 cells the fission yeast Schizosaccharomyces pombe (WT 972 h^- strain; kindly supplied by professor A. Goffeau, Louvain-La Neuve) is used. Two cell lines of this strain were available: the wild-type line and a mutant line (the red coloured Ad7 line).

1) Cell-survival studies.

As is reported in literature Schizosaccharomyces pombe cells, grown in EMM-medium to stationary phase, are in G1 or G2 phase of the cell-cycle, depending on the phosphate concentration in this medium. A low concentration (3mM) forces the cells into the G1-phase, whereas a high phosphate concentration (300mM) causes a population in G2-phase. Therefore the kinetics of radiation-induced cell-killing is studied in 4 types of cell population: wild-type cells grown to stationary phase in phosphate-rich and phosphate-poor medium and Ad 7 cells grown to stationary phase in phosphate-rich and phosphate-poor medium.
The survival curve for the 'phosphate-poor' cells - reportedly all in G1-phase - was complex and apparently consists of two different curves. This indicates that these populations are not homogeneous with respect to radiosensitivity.

Cytological investigation revealed that the cells were not uniform in size, even cell plates could be observed in some cells. In order to obtain a more synchronised culture, G1-cells were separated from the population by means of density-gradient centrifugation. This procedure resulted in a far more homogeneous G1-population, but the homogeneity with respect to rediosensitivity was not yet complete. Due to time shortage the synchronisation into G1-cells homogeneous in radiosensitivity, could not be fully worked out.
In contrast to the so called G1-cells, the G2-cells showed a perfect homogeneity with respect to radiosensitivity. The survival curve shows an initial shoulder followed by a sharp dicline at high doses; it can perfectly be described by the molecular theory of Chadwick - Leenhouts (the C-L-model).

2) Cell survival - mutation frequency relationship.

To study the survival - mutation frequency relationship predicted by the C-L-model, only the Ad7 cells were used, grown to stationary phase in phosphate-rich medium. The reasons therefore are:
a) The Ad7 population, grown to stationary phase in phosphate-rich medium shows uniform radiosensitivity.
b) As compared to the wild-type cells, grown to stationary phase in phosphate-rich medium, the red-white mutation ocurred much more frequently than the white-red mutation.
c) After irradiation of wild-type cells, grown to stationary phase in phosphate-rich medium, a reddish coloured mutant occurred with rather high frequency. Therefore the frequency of mutation white-red was never unambiguously scored.

When plotted against the log of survival the mutation frequency red-white consistantly shows a straight line, as far as the mosaic formation is concerned. On the contrary, the formation of complete mutants showed different curves - including a straight line - in all experiments.
So, whereas the formation of mosaic-mutations is in complete accordance with the predictions, made by the molecular theory of Chadwick and Leenhouts, the discrepancy between the theory and the formation of complete mutants remains to be explained.
A more detailed record of the experiments is given in the Internal Report no. 156.

C. Experiments with Haplopappus gracilis (Nutt.) Gray.

1. Seeds of Haplopappus gracilis (obtained from professor R. Tanaka, Hiroshima, Japan and dr. H. Smith, Brookhaven, U.S.A.) were germinated in the greenhouse under standard conditions (16 hours light, 8 hours darkness; 26°C; r.h. = 60%). The plants grew to the flowering state within 4 months and seedsetting occurred. The viability of that seeds was tested by sowing them. Only about 5% of the seeds germinated and grew into the flowering state.
Since that new population as well as the original population shows a great variability with respect to shape, color, growth rapidity etc., it was decided to see if cloning was possible. A fast growing plant was chosen and cuttings were placed in Homes' nutrient solution. Within 14 days most of the cuttings developed roots and after 2 months grew into normal plants.
Such a clone of H. gracilis is currently in use for experiments.

2. Starting from numerous techniques, recently presented in literature for both animal and plant material, a chromosome-banding technique, especially adepted for H. gracilis, has been developed.

In short the technique contains the following steps:

a) Young clean root tips are fixed in Alcohol-acitic acid solution (v/v = 3/1) during a minimum of 24 hours.
b) After several washings in 70% alcohol, the fixed root tips are hydrolized in N.HCL (4^1; 60°C) and successively washed in 70% alcohol and H_2O.
c) The hydrolized root tips are squashed in 45% Acetic acid. The coverslip is removed after freezing and the preparation is quickly air dried at a temperature of 40-50°C.
d) The preparation is incubated in saturated $Ba(OH)_2$-solution at 60°C for 15 minutes and immediately there after incubated in double concentrated standard Saline Citrate solution at 60°C for 60 minutes.
e) The preparation is stained in Giemsa stain-solution (Gurr) at room temperature for 90 minutes. After that staining the preparation is washed with 70% alcohol and mounted in Euparal.

As is shown in fig. 1, the technique gives a real longitudinal differentiation of the chromosomes.

In order to test the reproducibility of the technique and to produce a standard karyotype of H. gracilis, experiments using cloned plant material are in progress. Since it is very important to use clean root tips only the root tips of 12 days old cuttings are used for these experiments.

3. Callus and suspension cultures of H. gracilis were kindly supplied by dr. H.J. Fritsch, Freiburg i. Br., G.F.R. They are propagated awaiting the delivery of appropriate shakers to carry out experiments.

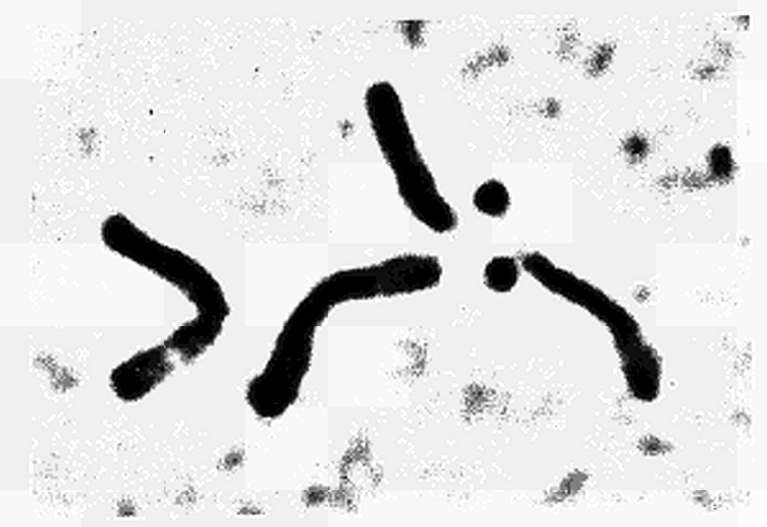


Fig. 1. Giemsa-stained metaphase chromosomes of Haplopappus gracilis. The longitudinal differentiation along the chromosomes is clearly shown. (3250x)

Resultaten van het project No. 10

Hoofd van het team en wetenschappelijke medewerkers:
K.H. Chadwick, K.J. Puite

Titel van het project:

Applied dosimetry

Beschrijving van de resultaten:

A. Additional EULEP dose intercomparisons

During the EULEP X-ray dose and dose distribution intercomparisons carried out in 1971 and 1973 absorbed dose values of one of the participating institutes were found 15 - 20 % lower than those obtained at the standardization laboratory. Evidence was available indicating that the dosimetry of the 15 MeV X-rays used at this institute was reasonably accurate and, although a 3% error was found due to an incorrect value of the rad/R factor used no explanation of the discrepancy in dose could be found. Additional dosimetry measurements which also included the ^{60}Co gamma ray and 250 kVp X-ray facilities at this institute were carried out. The results of the ^{60}Co gamma rays and 250 kVp X-rays were quite satisfactory but those of the 15 MeV X-rays show a large variation of the dose in time and space in the radiation field. This variation coupled with the error due to the rad/R factor could explain the discrepancy in absorbed dose values obtained during the intercomparison.

B. Thermally stimulated current measurements

The measurement of the radiation induced thermally stimulated current (TSC) in suitable materials offers an alternative method of dosimetry with certain advantages (simple and inexpensive apparatus, no photomultiplier). A preliminary set-up of such a system has been made, using BeO as the dosimeter material. Absorbed doses from 0.2 to 25 krad have been measured. The TSC signal increase is less than linear with dose. Technical difficulties with the electrical contacts and with the heating system make this system less attractive for further development.

C. Lyoluminescence measurements

When some solids, after being irradiated, are dissolved in water a light emission takes place. In general, the light signal is proportional to the absorbed dose in the solid. According to recent literature also some saccharides exhibit this lyoluminescent phenomenon. As these materials are cheap and nearly tissue equivalent, having an hydrogen content of about 7%, they may be used as throw away dosimeters in X-ray and fast neutron fields. Using a simple lyoluminescence apparatus, measurements have been performed with mannose, xylose, trehalose and glucose monohydrate samples from various manufacturers. Mannose samples gave the highest light output, which varied with the origin of the material. No fading of the signal has been observed during a storage time of 20-300 h after exposure.

The measurements will be repeated and extended and material will be selected for application in fast neutron dosimetry.

D. Climate box for high dose level exposure.

On request of the food technology group a prototype of a climate box is being built, which will be used for radio-biological experiments in a high dose level γ-field. Materials with small radiation damage had to be selected. A fixed temperature between -80°C and +80°C can be chosen with the aid of a liquid nitrogen cooling system and a heating system. It is foreseen that this climate box can be used also by other research groups.

E. High level gamma and electron dosimetry

Following the reloading of the ^{60}Co-source at the Pilot Plant for Food Irradiation, new dose measurements were made using clear perspex dosimeters. New calibrations of the special calibration positions were made using the Fricke dosimeter. Initial dosimetry measurements were made for the electron irradiation of sludge a 3 mm perspex build up layer and a 4 mm layer of slib with aluminium backscatter gave a uniform irradiation. Dose and dose distribution measurements were also carried out in 'Gammaster' for OPG following reloading. Some small deviations in the estimation of dose rate have been found using the low dose portion of the clear perspex calibration curve and as this part of the curve is dependent on the oxygen content of the unirradiated perspex, some attention is being paid to the stabilisation of this part of the curve. Measurements with 3 mm red and amber perspex have shown that:

1. 3 mm amber is too thick for measurements of more than 2 Mrad
2. in amber perspex the 603 nm peak is unaffected by heating up to 55°C, the 651 nm peak fades slightly; above 80°C both fade and at 150°C the signal fades very rapidly in less than 1-2 min.
3. in red perspex the OD spectrum changes on heating up to 80°C and it may be possible to find one wave length giving good stability up to this temperature. At this temperature, the peak at 600 nm develops extensively. At 150°C the signal fades rapidly. The higher temperature stability of the red perspex may be an advantage in special irradiation conditions.
4. The radiation induced signal fades on oxygen diffusion almost completely, showing a similar behaviour to the clear perspex.
5. The OD-dose response curve in red and amber has almost exactly the same form as the unstable OD (free radical)-dose response in clear perspex.

Dose measurements in the Bhabha Atomic Research Centre in Bombay, India have indicated that, although the various perspex dosimeters can be accurately and reproducibly calibrated under certain irradiation conditions, e.g. long irradiation time (80 h), above normal temperatures, certain effects may occur, e.g. OD spectrum changes, O_2 diffusion fading etc. which can lead to systematic errors even though the reproducibility remains good. This confirms what has been suspected for some time. The measurements also indicated that the new batch III H X clear dosimetry perspex was unsatisfactory as a dosimeter system and also that a 2 mm thick perspex sample would combine optimal properties of minimal fading and good OD level for use in a radiation sterilization plant.

Publications 1974

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J.J. BROERSE and K.J. PUITE,
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K.J. PUITE, G. SCARPA and J.J. BROERSE,
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K.J. PUITE and D.L.J.M. CREBOLDER,
The use of thermoluminescent materials for dose and dose distribution intercomparisons in X-ray fields. Newsletter on the Application of Nuclear Methods in Biology and Agriculture 3, 33 (1974) (ed. Ass. Euratom -ITAL, Wageningen).

K.H CHADWICK,
Dosimetry techniques for Commissioning a Process. In Sterilization by ionizing Radiation: Technical Developments and Prospects (ed. Gaughran and Goudie). Multiscience Publications Ltd. Montreal 1974.

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1. Solid State Dosimetry at High Doses
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Papers presented at the International Course on Ionizing Radiation Metrology held in Varenna, Italy, Oct. 1974.

K.H. CHADWICK,
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K.H. CHADWICK,
A standard procedure for the use of the clear perspex HX Dosimeter in the dose range 1-10 M rad. In Experiences in Radiation Sterilization of Medical Products. IAEA-159 (Vienna) 1974. pp 99-114.

Contrat N°:SC 010/094-72-1 BIA N
Université Catholique de Louvain
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1348 Louvain-La-Neuve.

Prof.H.LAUDELOUT

List of scientists having contributed to this report :

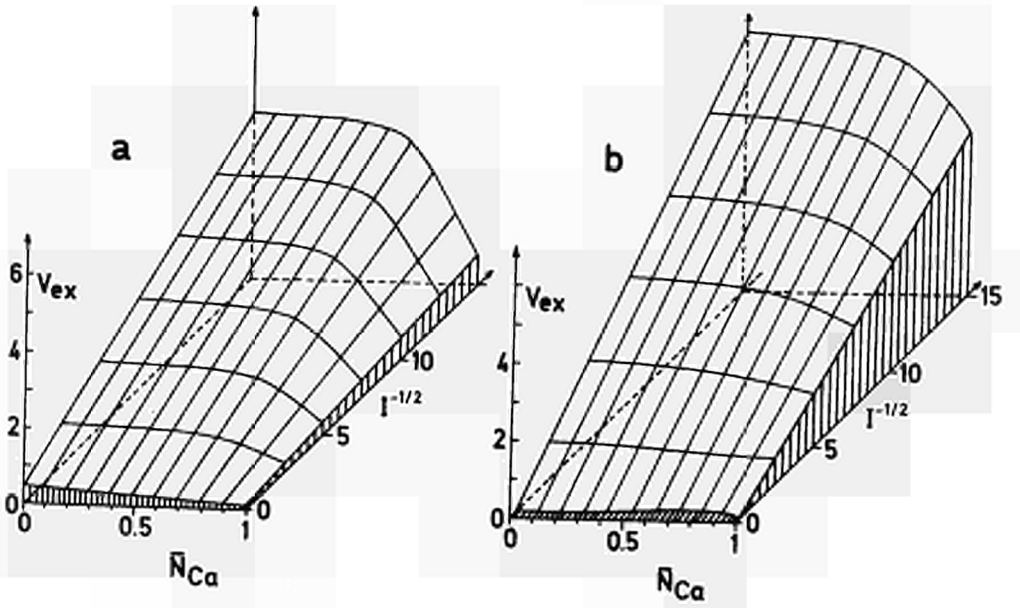
Prof.R.Van Bladel, Prof. R.Lambert, Dr.Tang Van Hai, Dr.J.Dufey, Mr.A.Moreale.

Thème général du contrat : Movement of soil ions and their uptake by plants.

Project Nr.1 : Ion Movement and Exchange in Soils.

Further work has been done on the numerical aspects of a model describing the movement of three cations through a layered soil at constant fluid velocity. Since this model is now operating satisfactorily, the next step will involve the interface between its subroutines describing ion exchange isotherms and a main programme describing water movement in unsaturated conditions, many of those are currently available.
If the movement of solutes such as cations often can be described satisfactorily without too much attention being paid to kinetics of the processes, this is never the case for mineral or organic nitrogen compounds moving through the soil where kinetic relationships only will be used for modeling the transfer of solutes. Some other solutes occupy an intermediate position in that respect such as pesticide molecules the movement of which can only be described in terms of adsorption equilibria and rates.
For these reasons an effort was made in order to arrive at a better knowledge of the parameters involved in these movements of nitrogen and pesticide compounds. Since the oxidation of ammonium or nitrite implies generally growth of the bacteria coupled to their oxidative activities, a knowledge

of the molar growth yield is important.
Furthermore the kinetic parameters of the various oxidative and growth equations for the oxidation of ammonium by mixed cultures have been incorporated into a submodel which has been run at various temperatures with the result that the transient concentration of nitrite becomes noticeable at high temperature only provided chemical denitrification favored by low moisture, high colloid content and low pH does not compete with biological oxidation with the results that loss of nitrogen occurs . Experimental confirmation of this has been obtained and further work is being done on the parameters of the denitrification reaction.
Work has been carried out on the adsorption equilibria of several pesticide molecules prior to the study of their convective transport in soil columns.
The fundamental aspects of the solute transport in soils have been investigated from the following point of views :
Three uni-univalent mixed cation clays have been studied for determining the effect of the ionic environment on the mobility of Na^{+} on clay surfaces .
It was shown that the mobility was increased when the other compensating cation was of higher polarisability and therefore excluded more or less completely Na from the Stern layer. When the other compensating cation is Ca^{++}, a case of more interest pratically, different relationships are observed introducing Ca^{++} modifies only slightly Na^{+} mobility up to a calcium loading of about 40 to 50%.
Similar relationships have been observed on the effect of increasing Na loading on properties related directly or indirectly to transport processes in soils. This is the case for the exclusion volume which is related to the pore volume available for the movement of anions. This rather curious and important relationship is illustrated in the figure.
As evidenced by the figure, our observations are very well related with on the one hand predictions from diffuse double layer theory and on the other hand empirical knowledge of field agronomists on the effect of exchangeable sodium percentage.



Three-dimensional diagrams relating the exclusion volume, V_{ex} ($ml.g^{-1}$), to the ionic strength, I, and to the equivalent ionic fraction of adsorbed Ca^{++}, $\bar{N}_{Ca}$: a - experimental best fit
b - double layer theory.

Project Nr.2 : Uptake of Solutes by Plants from a Dilute Environment.

The studies carried out previously on nitrogen uptake by rice as influenced by P, K, Ca and Mg concentrations were continued. It was observed that the ammonium absorption curve was of the dual mechanism type at all stages of growth i.e. 25, 50, 100 and 130 days. The K_m value being about one order of magnitude smaller (0.5×10^{-4}M) in the low concentration range than in the high concentration range. Efficiency of N uptake in continuous flow culture was related to K content with even slight ammonium toxicity effects at low K content.

The same technique was used for studying the absorption of herbicides by intact rice plants.

The results have been presented at length in the paper mentioned in the list below.

The uptake by intact rice plants was measured at concentrations varying from 0.001 to 3 ppm 2.4 D and 0.005 to 2.5 ppm 2.4.5.T . A comparison was made of the kinetic analysis of the uptake curve according to the Michaelis or the Thellier formulation.

For instance the four parameters occurring in Thellier's equation :

$$V = 2.3 \frac{A}{r} \log \frac{B}{(P)} \cdot (S) + 2.3 A \log \frac{B}{(P)} (S)^m$$

had the following values :

	2.4 - D	2.4.5. T
B/(P)	276	95
r	945	1540
m	3.74	4.68
A	0.2	0.4

while the K_m for 2.4.5.T and 2.4.-D was about 3 µM while the V_m was from 0.1 to 0.3 $\mu M.h^{-1}$ per g. dry weight.

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-LAUDELOUT,H., LAMBERT,R., FRIPIAT,J.L. et PHAM,M.L. Effet de la température sur la vitesse d'oxydation de l'ammonium en nitrate par des cultures mixtes de nitrifiants.
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-DUFEY,J.E., and LAUDELOUT,H.G. Hydration numbers of Na-Ca montmorillonite.
Soil Science.(soumis pour publication).

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Journal of Colloid and Interface Science.(soumis pour publication).

Université Catholique de Louvain
N° 096-72-1-BIO B
A. GOFFEAU
Transport of radionuclides by biological membranes

Although the move of the laboratory from Leuven to Louvain-la-Neuve has slowed down our research in 1974, substantial progresses have been made for the two research projects. In both cases, the use of yeast as a model eucaryot cell has permitted a variety of physiological, biochemical and genetical approaches.

1. The stimulation by strontium of the mitochondrial oxidation of external NADH discovered last year has been confirmed. The site of the action of strontium has been further precised. The stimulation appears to be due to a firmly membrane-bound enzyme involved in the mitochondria oxido-reduction chain reducing ferricyanide from external NADH. This enzyme is located between NADH and the site of action of antimycin A and might be the external NADH dehydrogenase of the inner mitochondrial membrane. A purely chemical modification of the redox properties of NADH by strontium has also been discovered and characterized. Genetical modification of the stimulation by strontium of NADH: ferricyanide oxidoreductase activity has also been observed and the pleiotropic properties of these mutants have been thoroughly investigated (references 1 to 9).

2. An active transport of strontium and calcium by Schizosaccharo-pombe has been discovered and characterized. The effects of time, pH, temperature, substrates, respiratory inhibitors and uncouplers have been thoroughly studied. It was concluded that strontium as well as calcium are transported by two distinct classes of saturable "carriers" with either low or high affinities. The transport requires metabolic energy produced either by fermentation or respiration. An unexpected stimulation of uptake by uncouplers has

been observed and is not understood presently. Strontium and calcium compete for the same carrier with apparent similar affinities while magnesium is about ten times less strongly bound. Unlike the transport of amino acids, nucleosides and other metabolites, the active uptake of strontium under starvation condition is not stimulated by the addition of exogenous cyclic AMP. A membrane-bound non-mitochondrial ATPase activity, possibly involved in active transport through the plasma membrane has been identified, partially purified and characterized. This ATPase is however not stimulated either by calcium, strontium, potassium or sodium but requires magnesium, manganese, zinc or cobalt for maximal activity (references 10 to 13).

Publications

1. A. GOFFEAU, A.M. COLSON, Y. LANDRY, F. FOURY and M. BRIQUET
Stable nuclear Pleiotropic Respiratory-Deficient Mutants with modified Mitochondrial Cytochrome a+a_3 and Adenosine Triphosphatase in a "Petite-Negative" Yeast, Schizosaccharomyces pombe.
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2. A. GOFFEAU, M.F. LABAILLE, O. MOHAR and A. TZAGOLOFF
Common control by a Single Gene of the Syntheses of Cytochrome Oxidase and Oligomycin-Sensitive ATPase.
Hoppe-Seyler's Z. Physiol. Chem. 355, 29 (1974)

3. A.M. COLSON, A. GOFFEAU, P. WEIGEL, G. RANK and J.R. MATTOON
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4. A.M. COLSON, C. COLSON and A. GOFFEAU
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5. A. GOFFEAU, A.M. COLSON, Y. LANDRY, F. FOURY and M. BRIQUET
Stable Pleiotropic Chromosomal Mutations with Modified Mitochondrial ATPase and Cytochromes aa_3 and b in Schizosaccharomyces pombe.
In Biomembrane, Architecture, Biogenesis, Bioenergetics and Differentiation, ed. by L. Packer, 35-48 (1974) Acad. Press.

6. Stable pleiotropic respiratory-deficient mutants of a "petite-negative" yeast : Schizosaccharomyces pombe as a new tools to study the nuclear control of the assembly of the inner mitochondrial membrane.
A. GOFFEAU, A.M. COLSON, J. DELHEZ, F. FOURY, F. LABAILLE, Y. LANDRY, O. MOHAR and E. MRENA
In Membrane Biogenesis : Mitochondria, Chloroplasts and Bacteria, ed. by A. Tzagoloff, Plenum Publishing Corporation (in Press).

7. André GOFFEAU, Françoise LABAILLE and Anne-Marie COLSON
Pleiotropic modifications in a mutant of Schizosaccharomyces pombe lacking oligomycin-sensitive ATPase.
In "Nucleocytoplasmic Relationships during Cell Morphogenesis in some unicellular Organisms" ed. by Puiseux-Dao, Elsevier Scientific Publishing Company (in press).

8. Y. LANDRY and A. GOFFEAU
Physiological and genetic modification of the expression of the yeast mitochondrial adenosine triphosphatase inhibitor.
Biochim. Biophys. Acta (in press).

9. A.M. COLSON, A. GOFFEAU, M. BRIQUET, P. WEIGEL and J.R. MATTOON
Nucleo-cytoplasmic interaction between oligomycin-resistant mutations in Saccharomyces cerevisiae.
Molecular and General Genetics (in press).

10. F. FOURY and A. GOFFEAU
Stimulation of cellular transport by cyclic AMP in yeast.
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11. F. FOURY and A. GOFFEAU
Stimulation of Active Transport in Yeast by Cyclic AMP.
Abstracts 9th FEBS Meeting, Budapest, Abstract s6 é22, 255 (1974)

12. Françoise FOURY and André GOFFEAU
Stimulation of active uptakes of nucleosides and amino acids by cyclic adenosine 3',5'-monophosphate in yeast.
J. Biol. Chem. (in press)

13. Françoise FOURY
Effects of cyclic AMP on yeast plasma membrane functions.
In "Nucleocytoplasmic Relationships during Cell Morphogenesis in some unicellular Organisms" ed. by Puiseux-Dao, Elsevier Scientific Publishing Company (in press).

Résultat du projet n° 1
Chef du projet : A. GOFFEAU
Collaborateurs scientifiques : M. BRIQUET, A.M. COLSON, M.F. LABAILLE

Transport of radionuclides by yeast isolated mitochondria

The stimulation of the mitochondrial oxidation of NADH by strontium and calcium which was discovered last year with ethanol-grown Saccharomyces cerevisiae intact mitochondria, could have been interpretated as an indication of an active calcium and/or NADH transport by yeast mitochondria. This possibility is now very unlikely since the stimulation has also been obtained in sonicated submitochondrial particles where the permeability barrier is destroyed. The stimulatory effect of strontium has been restricted to the cyanide and antimycin A-insensitive NADH: ferricyanide oxidoreductase activity. Since the stimulation is observed in intact mitochondria impermeable to external NADH, as well as in sonicated submitochondrial particle, the site of action is likely to be the external NADH dehydrogenase of the inner mitochondrial membrane. Solubilization of the NADH: ferricyanide oxido-reductase is underway.

It has been discovered that the chemical reduction of ferricyanide by NADH is also stimulated by strontium, calcium and magnesium. This stimulated chemical oxido-reduction which exhibit two peaks of optimal pH: 4.4 and 6.5, suggest the existence of chemical complexes of strontium (or other divalent cations) with NADH. These complexes are more reactive both chemically and enzymically than NADH.

The enzymatic stimulation is not specific to ethanol grown Saccharomyces cerevisiae since it has also been observed with glucose grown Schizosaccharomyces pombe mitochondria. In the latter species, we have obtained and studied a serie of single nuclear gene respiratory-deficient mutants. The mutant M126 exhibits a modified mitochondrial ATPase which is insensitive to oligomycin. Although this strain exhibits several other pleiotropic modifications of the respiratory chain, a weak NADH: ferricyanide oxidoreductase activity is still observed. This activity

is no longer stimulated by strontium. Functional respiration is thus notnecessary for the stimulatory effect. However the introduction of an additional mutation restores the stimulation by strontium of the NADH: ferricyanide activity. It is remarkable that the double mutant is still respiratory deficient. It is hoped that further analysis of the primary gene products producing these pleiotropic modifications will throw some light on the physiological meaning of the stimulatory effects of strontium and calcium.

Projet n° 2
Chef du projet : A. GOFFEAU
Collaborateurs scientifiques : J. DELHEZ, F. FOURY et E. MRENA

Role of the plasma membrane in the transport of radionuclides

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Glycerol-grown Schizosaccharomyces pombe cells put under glucose starvation take up ^{45}Ca at constant rate for about 30 min. At pH 4.5, the Vmax for the uptake is about 0.1 nmoles ^{45}Ca per min and per 10^8 cells. The Vmax increases with pH and reaches 0.50 picomoles per min per 10^8 cells at pH 8.5. At both pH, biphasic kinetics producing two apparent K_M are distinguished : 80 µM and 530 µM at pH 4.5 and 30 µM and 220 µM at pH 8.5. Active transport of calcium is inhibited competitively by the addition of strontium suggesting that both cations are transported by the same carrier. Magnesium however is much less active: 0.08 mM strontium increases the K_M for calcium from 33 µM to 83 µM, while 0.84 mM magnesium is needed to produce a similar ΔKm. Under alcaline condition the ^{45}Ca uptake is energy dependent. In the presence of 1.2 µM $CaCl_2$, the addition of 70 mM glucose increases the ^{45}Ca uptake from 1.33 to 5.98 picomoles per min and per 10^8 cells. In the absence of glucose, the respiration of endogenous substrates (160 µl O_2 per hr per 10^8 cells) furnishes the metabolic energy required for the uptake as demonstrated by the effects of respiratory inhibitor. The uptake decreases from 6.86 to 0.58 picomoles ^{45}Ca per min and per 10^8 cells after addition of 2 µM antimycin A. Quite unexpectedly proton translocators (uncouplers) such as phenylhydrazone (CCCP) derivatives stimulate the uptake. At pH 8.5, in the presence of 100 mM glucose, the ^{45}Ca uptake increases from 0.66 to 2.56 picomoles per min and 10^8 cells after addition of 40 µM CCCP. The antibiotic Dio-9, inhibitor of plasmic and mitochondrial ATPase, produces similar stimulation at pH 4.5 as well as pH 8.5 even when the respiratory energy is blocked by the addition of antimycin A. These effects suggest that the driving force for the calcium and strontium uptakes is a pH gradient accross the plasma membrane with the alcaline pH at the outside. This driving

force is opposite to the one required for the uptake of amino acid and other metabolites for which the ΔpH is acid outside. The two types of transport are also distinguished by the effects of cyclic AMP which stimulates the uptake of amino acids but does not modify that of strontium or calcium.

To further analyse at the molecular level the mechanism of transport of strontium and calcium, a method for purification of yeast plasma membranes has been elaborated. The purified plasma membrane fraction contains an ATPase activity which can be distinguished from the mitochondrial enzyme by its optimal pH which is 6.0 compared to 8.5 for the mitochondrial enzyme. The plasma ATPase has been solubilized with lysolecithin and partly purified. The enzyme activity requires magnesium, manganese, cobalt or zinc but is not stimulated by calcium or strontium suggesting that this enzyme is not directly involved in the transport of the latter ions.

Annexe administrative

1. Le Laboratoire d'Enzymologie a déménagé en novembre 1974 de Leuven et occupe actuellement une surface de 300 m^2 dans l'Unité de Biochimie Physiologique de la Faculté des Sciences Agronomiques de l'Université Catholique de Louvain à Louvain-la-Neuve.

2. Le professeur A. Claude du Laboratoire de Biologie Cellulaire associé au Laboratoire d'Enzymologie a obtenu le Prix Nobel en octobre 1974. Certains travaux de morphologie effectués dans le cadre des travaux du programme EURATOM : UCL n° 096-72-1-BIO B ont été effectués dans le laboratoire de professeur A. Claude.

Contractor: United Kingdom Atomic Energy Authority

Contract No.: 133-74-1 B10 UK

Head of research team: Mr. A. Morgan

General subject of contract: Uptake of tritium from accelerator targets

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The evolution of tritium from two used neutron generator targets has been studied. One target had been used and stored for seven months before measurements were made and the other was removed from the generator and examined forty-eight hours after its last bombardment.

Room air containing 0.5 per cent of hydrogen was passed over the targets at 1 l min^{-1}, corresponding to a linear flow rate of 0.5 cm sec^{-1}. The resultant gas was analysed for particulate tritium, tritiated water and tritium gas. Particulate tritium was removed by a 0.8 μm Millipore filter. Tritiated water was separated from tritium gas by absorption in three water bubblers. Tritium gas was oxidised by passage over a heated platinum catalyst and then removed by further water bubblers. The tritium content of the water from the bubblers was determined by liquid scintillation counting. The tritium content of the Millipore filters was determined by combustion and subsequent liquid scintillation counting of the water produced.

In order to study the particles detached from a target by mechanical shock, an apparatus was constructed similar to that described by Fehér and Biró[1]. The target was fastened, with its active face downwards, to a horizontal piston. The piston was propelled by a spring into a vertical tube where it stopped by impact. Particles detached from the target by deceleration forces fell into the tube which was connected to a cascade centripeter[2] sampling air at a flow rate of 30 l min^{-1}. Particles were collected in four ranges:- > 12.5 μm, 12.5-4.0 μm, 4.0-1.5 μm and < 1.5 μm. The tritium activity associated with each size range was determined by combustion and liquid scintillation counting.

Results of Project:

Head of Project and scientific staff: J. D. Eakins
A. E. Lally

Title of Project: Internal contamination with tritium arising from the use of tritium-titanium targets in neutron generators.

Results

The total evolved tritium from the stored target was 1.2 μCi Ci^{-1} day^{-1}, of which 1.1 μCi was as tritiated water, the balance being mainly due to tritium gas. The particulate tritium was only 0.005 μCi Ci^{-1} day^{-1} and this could well have been due to water vapour adsorbed on the filter. In contrast, the recently used target initially evolved tritium at a rate of 11.6 μCi Ci^{-1} day^{-1} of which 11.2 μCi was tritiated water and the remainder tritium gas. However, as can be seen from Fig. 1, the tritiated water evolved decreased by a factor of 2 in 30 days whereas the tritium gas fell by a factor of 7 in the same time. As this target was initially examined 48 hours after its final bombardment, the results suggest that much higher levels of tritium are released immediately after bombardment and, in particular, the proportion of tritium gas will be much greater.

At low air velocities, little if any particulate material is removed from the targets. Some particles were obtained from the fresh target by smearing and the activity determined by suspension in liquid scintillator. The tritium content of the particles was also determined by combustion followed by liquid scintillation counting. The activity as determined by the combustion technique was on average a factor of 2 greater than that determined by suspension counting.

Particles collected by the centripeter were examined microscopically and found to be flakes of irregular shape. The mean Martin's diameters of each fraction were 14.7 μm, 7.1 μm, 3.4 μm and 2.1 μm, in close agreement with the mean aerodynamic diameters.

The activity distribution between the particle fractions was measured on four successive runs and the results are shown in Table I as

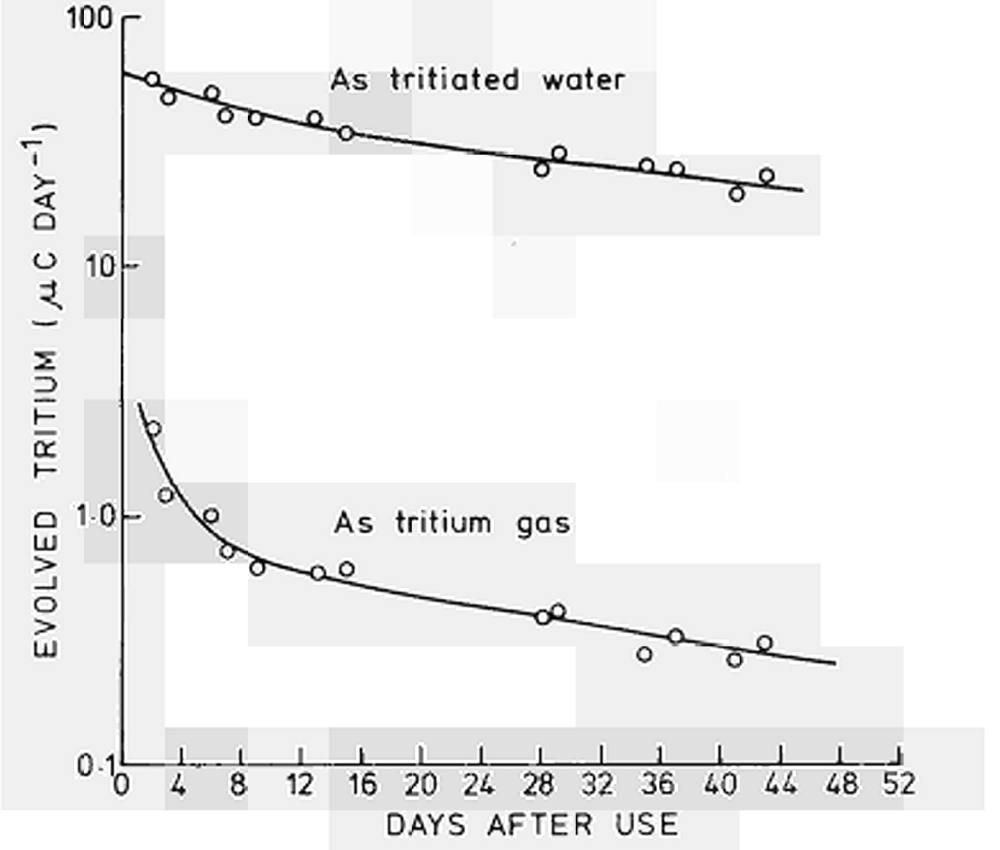

Fig. 1. Evolution of tritium from a tritium/titanium target bombarded 48 hours prior to the first measurement.

percentages of the total activity collected.

Table I

Activity distribution of particles from a tritium/titanium target

Run No.	Mean aerodynamic diameter			
	> 12.5 µm	12.5-4.0 µm	4.0-1.5 µm	< 1.5 µm
1	93.34	0.43	6.11	0.12
2	88.00	6.78	3.86	1.36
3	99.31	0.06	0.53	0.10
4	99.72	0.15	0.07	0.06
	95.09	1.86	2.64	0.41

The bulk of the tritium is associated with the larger particles and there is considerable variation in that associated with the smaller particles. However, it is possible to obtain a significant fraction in the respirable range, a fact which was not observed by Fehēr and Birō in their study with unused targets.

It is proposed to repeat the above experiment with an unused target to determine the activity distribution of the particles produced. The evolution of tritium from a target removed from a neutron generator immediately after bombardment will be studied and percutaneous absorption and ingestion experiments will be carried out using particles produced from such a target.

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Contractor: Ministry of Agriculture, Fisheries and Food
Lowestoft, Suffolk NR32 1DA

Contract No: 137-74-7 BIO UK

Head of research team: Dr. N.T. MITCHELL

Project 1

Transport and distribution of transuranic radionuclides in the marine environment following waste disposal from fuel reprocessing

Several transuranic nuclides have recently been attracting increasing attention in the context of their environmental impact and behaviour, particularly those of plutonium and especially the alpha emitters plutonium-239 and -240, which are being produced in increasing quantities as the use of nuclear power expands. Further interest is attached to these nuclides because of the development of fast breeder reactor systems containing plutonium as a fuel and also because of their unusually long radioactive half-lives. The next most important alpha-emitting transuranic nuclide is americium-241, the daughter product of yet another plutonium isotope, the β-active plutonium-241. Its significance is heightened by a relatively long half-life (458 years) which though shorter than those of the alpha-active plutonium isotopes, is of such a length that it represents a potential risk to many future generations.

Controlled discharges of low-level liquid radioactivity from the BNFL fuel reprocessing plant at Windscale contain each of these nuclides and present a valuable opportunity for studying their environmental behaviour and distribution in the marine environment. The north-east Irish Sea has become well labelled in recent years as a result of receiving these nuclides which can be detected in a wide range of materials - sea water, suspended matter, biota (fish, shellfish and algae) and bed sediment.

The Laboratory has already been active in this area for some years and has amassed a data base on which the Windscale effluent has been evaluated and controlled. The work being done under this contract will provide for extension of these studies and evaluation of long-term problems in the marine environment at levels subcritical to the present day public health risks.

Some extensive sampling has already been undertaken from a research vessel cruise in the Irish Sea taking sea water, suspended matter and bed sediment by both grabbing and coring. This has been supplemented by samples of fish, shellfish, the seaweed Porphyra and estuarine sediment. Analysis of these samples is now underway after some initial work on analytical techniques, especially americium. Both chemical and direct radiometric techniques are being employed as appropriate, the latter by counting X-ray emissions with thin NaI(Tl) crystal detectors.

Project 2

Transport and distribution of fission-product radionuclides in the marine environment following waste disposal from fuel reprocessing

Of the many fission-product radionuclides which enter the marine environment due to waste disposal following the generation of electricity by nuclear power, those of caesium-134 and -137 have emerged as being the most important in a radiological sense. Important features are half-life (especially in the case of caesium-137), a relatively conservative behaviour in sea water so that a significant proportion from a specific release may travel long distances in the seawater compartment and a small but important interaction with fish and shellfish through which they carry a potential public health significance.

This importance of caesium-134 and -137 has been established over a number of years past through work done at the Laboratory in support of the control of low-level liquid radioactive waste disposal from the BNFL fuel reprocessing plant at Windscale. This has established the importance of several compartments of the marine ecosystem - sea water, biota and sediment - especially in relation to local contamination problems and the critical factors which dictate limitations on effluent release.

More recently the Laboratory has turned attention to longer-term effects in respect of both time and distance, and the work being done under this contract is part of this further research from which problems occurring elsewhere due to caesium can be evaluated.

Use is being made of changing distributions of caesium as a result of variations in effluent discharge rates. It is hoped that the ratio of caesium-137 to caesium-134 can be utilized to establish transit times comparable to the half-life of caesium-134. Samples of sea water, sediment and biota (especially fish and shellfish) are being analysed by gamma spectrometry. This work is continuing and will be related to parallel work on sediment interaction.

GENETISCHE STRAHLENWIRKUNGEN

HEREDITARY EFFECTS OF RADIATION

EFFETS HEREDITAIRES DES RAYONNEMENTS

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

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

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

094-BIAN ITAL, Wageningen (De Zeeuw)

Contractor: Professor K. A. Marcker, Department of Molecular Biology, University of Aarhus, Denmark

Contract No.: 122-74-1-Bio-DK

Head of research team: Dr. Ole Westergaard

General Subject of Contract: DNA repair/DNA replication

In order to study the reactions of cells to damage of DNA by radiation, carcinogens etc., we feel it is necessary to develop procedures for isolating specific chromatin structures, before a complete understanding of the effects of such treatments is possible. Thus the aim for our work is to purify such structures and study their composition and possible changes after the treatment of the cells with various DNA damaging agents.

Results of Project No.:	122-74-1-Bio-DK
Head of Project:	Dr. Ole Westergaard
Coworkers:	Dr. Brian Johnson, Dr. Johan Chr. Leer and Dr. Peter Piper
Title of Project:	The Effect of Radiation on the Discontinous DNA Replication by RNA Primed DNA Fragments in an Eukaryotic Organism

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Exposure of the eukaryotic organism Tetrahymena pyriformis to radiation, thymine starvation or treatment with Ethidium Bromide cause induction of a mitochondrial DNA polymerase in response to damage of DNA. A similar phenomenon might occur in the nucleus, where we find accumulation of a multi-enzyme-nucleic acid-complex after damage of DNA. The complex consists of at least three enzyme activities (a DNA polymerase, a RNA polymerase and a deoxyribonuclease) in addition to a DNA fragment of defined size. The complex is purified 1100 fold from the whole cells and all three enzyme activities copurify with the DNA fragment. The fragment accounts for about 0,2% of the total genome. Electron micrographs of the purified complex demonstrate a ball-like structure with a diameter of 200 Å. We have investigated several physiological functions of the isolated complex.

It has for example been investigated if the complex is part of a"replicative DNA intermediate", which we have found is accumulating in Tetrahymena after the above mentioned treatments. "Intermediate DNA" has been studied by density labelling experiments in CsCl gradients, where it bands as a distinct peak of intermediate density. This DNA contains single-stranded regions and after short pulses (<10 min) accounts for about 90% of the newly synthesised DNA. Longer pulses give in addition to the intermediate DNA, a DNA banding at the position of fully hybrid DNA. Treatment with single-stranded specific deoxyribonuclease converts a proportion of the intermediate DNA to

molecules of full hybrid density. Studies of the intermediate DNA in alkaline buoyant density and alkaline sucrose velocity gradients clearly demonstrate the appearance of non-covalent linked nascent synthesised fragments. So far, however, we have no clear evidence that the complex is part of the "replicative DNA intermediate", which is accumulating after DNA damage. We are still continuing this line of approach, in order to find a definite physiological role of the complex.

Our success in isolating intact nucleic acid-protein complexes has lead us to investigate the possibility of purifying specific functional chromatin structures. We have concentrated on trying to purify the chromatin structure specifying the ribosome RNA genes, because of the ease of assaying such genes. By simple procedures we have succeeded in puryfying this structure more than 40 fold from total chromatin. At present we do not know the exact protein composition of this part of the chromatin but we propose to investigate this in the near future. If our success in this respect can be continued we consider this a major break-through since it will allow us for the first time to study the individual porteins associated with a specific gene.

Contractant van de Commissie: Leiden State University
Nummer van het contract : 102-72-1 BIAN
Hoofd van het researchteam : Prof. Dr. A. Rörsch
Algemeen onderwerp van het contract:

Molecular Mechanisms of the repair of DNA damage

Results

To understand the mechanism of mutation in eukaryotic cells and to know more about their effect on differentiation plant cells are used as a model-system because of their known property of totipotency. To this end we are studying:

a) the mechanism of the induction of the planttumor Crown gall by Agrobacterium tumefaciens in comparison with habituation of normal cells under tissue culture conditions,
b) mutation induction of diploid and haploid plant cells (protoplasts) in vitro by UV irradiation and chemical mutagens,
c) genetic modification of plant cells protoplasts by the introduction of exongenous DNA of known function,
d) fusion of protoplasts for somatic cell genetics and in order to obtain new plant varieties,
e) DNA repair in plant cells after UV- and other irradiations.

Most of the work on b, c, d and e was started in 1974.

Resultaten van het projekt no. 1

Hoofd van het team en wetenschappelijke medewerkers:

R.A. Schilperoort (hoofd)
A. Rörsch
R.F. Heyn
J.J.M. Dons
A.M. Ledeboer
E. Wurzer-Figurelli
G.H. Bomhoff (heeft het lab verlaten)
H. den Dulk-Ras
A.K. Hermans
H. Kester
L. Otten (Euratom fellowship, Orsay)

Titel van het projekt:

Mechanism of mutation in eukaryotic cells

Ad a. In collaboration with the group of Prof. Schell (genetisch Laboratorium, Rijksuniversiteit Gent, Belgium) it has been found that a large plasmid in A.tumefaciens is essential for the tumor-inducing ability. We suppose that the synthesis of the guanidine derivatives octopine and nopaline in Crown gall cells is directed by plasmid genes in these cells. These guanidine derivatives are neither found in normal tissues nor in habituated tissues having also a tumorous character. Crown gall tissues either synthesize octopine or nopaline dependent on the bacterial strain used to induce the tumor and not dependent on the host plant.
The same strain-specificity is found for the utilisation of either octopine or nopaline by A.tumefaciens. This property was found to be plasmid determined. The guanidine derivatives are important markers for Crown gall cells and can be of value in setting up a model system for the genetic modification of protoplasts by using A.tumefaciens plasmid (see c) and can also be used as markers in somatic cell hybridization experiments (see d).
Both guanidine derivatives seem to determine in a specific way, but different for both, the degree of differentiation capacity of the Crown gall tumors. By studying the metabolic pathways disturbed by the synthesis of the guanidine derivatives, information can be obtained about the processes which are essential in differentiation.

Determination of the nuclear DNA content of leaves and normal, habituated and Crown gall callus tissues of Nicotiana tabacum var. White Burley were performed using cytophotometry on feulgen stained preparations. Several

aspects concerning the reliability of the feulgen technique for DNA determinations were investigated. It was found that Crown gall and habituated tissues acquire almost the same DNA content which is significantly higher than in normal tissues. The results suggest a correlation between the acquisition of a special chromosome complement and the loss of phytohormone requirement resulting in autonomous growth.

Ad b, c, d and e. To investigate the relation between dosage effect (survival and thymidine dimer formation) and chromosome number, suspension cultures have been started from callus cultures of White Burley (normal and Crown gall) and haploid and diploid N.sylvestris. Several media have been tested out for good callus growth of leaf and stem tissues of the haploid and diploid plants. The same had to be done to obtain the suspension cultures. Using these cultures and different mutagens including UV we are now trying to isolate 5-BUdR, 8-AzaG- and 5-methyltryptophane-resistent lines which can be used for the selection of heterokaryons in somatic cell hybridization.

Our work on the isolation of White Burley and Wisconsin 38 mesophyll protoplasts has shown that the growth conditions of the plants and the isolation procedure followed is of tremendous influence on the viability of the protoplasts and their regeneration capacity. Knowing the different factors involved we are now trying to quantitate their influence on protoplast viability in order to establish a reproducible isolation procedure for leaves and for cells in suspension culture.

In connection with studies on dedifferentiation processes on the level of DNA, synchronous DNA synthesis was observed in isolated leaf protoplasts using thymidine incorporation.

Using our recently published micro-DNA-isolation-procedure work is in progress (in Orsay) on the kinetics of satellite DNA appearing in Nicotiana glauca pith explants during short cultivation on agar media (project in collaboration with Prof. Buiatti - Pisa).

Experiments intended to see whether ColE1-DNA can be taken up and expressed in protoplasts have shown that the methods currently available to detect either colicine or colicine-mRNA in protoplast-homogenates, are as yet not sensitive enough.

Publikaties in 1974

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2. J.J.M.Dons, M.Valentijn, R.A.Schilperoort and P.van Duyn "Nuclear DNA content and phytohormone requirements of normal, Crown gall and habituated tissues of Nicotiana tabacum var. White Burley" Exp.Cell Res. 89 (1974) 283.

3. G.H.Bomhof "Studies on Crown gall - A plant tumor. Investigations on protein composition and the use of guanidine compounds as a marker for transformed cells" Dissertatie Leiden 1974.

4. N.van Larebeke, G.Engler, M.Holsters, S.van den Elsacker, I.Zaenen, R.A.Schilperoort and J.Schell "Large plasmid in Agrobacterium tumefaciens essential for Crown gall-inducing ability" Nature 252 (1974)169.

5. R.F.Heyn, A.K.Hermans and R.A.Schilperoort "Rapid and efficient isolation of highly polymerized plant DNA" Plant.Sci.Lett.2 (1974) 73-78.

Contractant : State University of Leiden
Contract no.: 102-72-a 1 BIAN
Project no. : A.2
Director : Prof. Dr. A. Rörsch
Title : The identification of enzymes and genes which are involved in the repair of radiation damage in bacteria.

Summary

ATP-dependent nucleases have a function in genetic recombination and repair of radiation damage. Further studies on the ATP-dependent exonuclease from Micrococcus luteus have provided evidence that the enzyme binds to the DNA at a site which is strongly positively charged. The enzyme was also purified from E.coli and its activity compared with the enzyme isolated from a mutant of E.coli, which has the interesting property that it is UV-resistent but X-ray sensitive (Ror mutant).

The role of DNA polymerase I in repair processes was studied further by developing a system in vitro, which will make it possible to detect the different activities of the enzyme accurately.
A new project was started in order to study repair processes in a semi in vitro system. It appears possible in this system to complement for a genetic defect by adding the lacking enzymes to a suspension of cells which have been made semi permeable by toluene treatment.
From mammalian cells an endonuclease was purified acting on UV and γ irradiated DNA. The site of action of the enzyme in UV irradiated DNA is a photoproduct other than pyrimidine dimers. These photoproducts can also be induced in vivo. Purified repair enzymes were also used in studies not directly related to repair in collaboration with others. This made it possible to localize the genes of human Adenovirus 5 which are involved in transformation.
Enzymes were isolated and construction of E.coli strains are under way in order to get amplification of those genes which are important for repair and which are difficult to study by concentional methods.

Results of project A.2.1

Project leader : Dr. P. van de Putte
Research workers: Dr. B.W. Glickman
Dr. B. van Dorp (contract ended September 1974)
Drs. H.L. Heyneker. Dr. Silvia Bacchetti (contract ended September 1974)
Dr. C.A. van Sluis
Dr. H. Pannekoek
Title of project: Studies in vitro to elucidate the mechanism of repair processes

Progress report

It is generally assumed that ATP-dependent DNases are involved in genetic recombination. The following results were obtained from studies to clarify the mechanism of DNA degradation by ATP-dependent DNase from Micrococcus luteus: 1) The enzyme binds only to the ends of linear duplex DNA, a reaction which occurs in the absence of ATP. 2) Degradation of DNA starts only when ATP is present. The DNA molecules are degraded exonucleolytically in a one by one mode of action. 3) The DNA is degraded to oligonucleotides with a broad size distribution. The degradation products are partly single stranded and contain also double stranded regions. 4) The enzyme is inhibited by dextran-sulfate and actinomycin D. Dextra-sulfate binds to the enzyme, probably on the active site, where also the DNA is bound. The following model for the degradation of DNA by the ATP-dependent DNase from M luteus is postulated: The enzyme binds to the ends of a DNA molecule on a site which is probably strongly positively charged. In the presence of ATP the enzyme moves from this position along the DNA chain making ad random breaks in both strands, resulting in the digestion of the DNA into acid-soluble products and longer single- and double-stranded products. The energy of the ATP is probably used to move the enzyme along the DNA molecule.

In 1972 F.L. Graham and A.J. van der Eb developped a transformation system which act on in vitro cultured cells. They showed that human Adenovirus 5 DNA is infectious for susceptible cells and is able to induce stable transformation in non-susceptible cells. Only a small portion (about 5%) of the Adeno 5 DNA is responsible for transformation and from physical studies evidence was obtained that the transforming segment (T segment) was located on the left hand end of the Adeno genome. In collaboration with Graham and van der Eb we have localized the T segment on the Adeno genome with the help of repair enzymes as follows: Adeno 5 DNA was digested from the ends by the action of exonuclease III. Subsequently the free single stranded ends were digested by nuclease S1 resulting in DNA molecules which were shortened from the ends. When more than 1% of each end was degraded transforming activity diminished rapidly. We concluded from these studies that the T segment is located between 1 and 6% from the left end of the Adeno 5 genome. The T segment is large enough to code for 1 to 2 average-sized proteins, so that presumably not more than 2 viral genes are involved in transformation by Adeno 5 and these must be contiguous.

The regulation of gene expression is studied by making use of the transcription of the E.coli tryptophane (trp) operon in vitro. In the presence of purified RNA polymerase termination factor rho the 4 ribonucleoside triphosphates and the DNA of a trp transducing phage messenger RNA is made which is specific for the genes of the trp operon. A new mechanism has been found that regulates the transcription of E.coli in a positive way. It was shown, that a protein fraction from E.coli is able to de-blockade a transcription barrier just ahead of the first structural gene and enables RNA polymerase to make messenger RNA from the structural genes of the trp operon.

An endonuclease acting on DNA exposed to ultraviolet light or γ-rays has been extensively purified from calf thymus. The enzyme has a pH optimum at pH 7.0 - 7.5, acts with equal efficiency in the presence of EDTA or divalent cations (Mg^{++} or Ca^{++}), is inhibited by NaCl and tRNA and is inactivated by incubation at 50°C. Its molecular weight, determined by Sephadex chromatography or SDS-gel electrophoresis, is ± 30,000. The enzyme catalyzes the formation of single-strand breaks with 5'-phosphate termini in double-stranded DNA irradiated with ultraviolet or γ-rays. It does not act on unirradiated DNA or denatured DNA. Since in all these properties the enzymatic activity on ultraviolet- and γ-irradiated DNA behaved similarly and since the two activities cochromatographed in all systems used during purification, we conclude that they are associated with the same protein. The site of action of the enzyme in ultraviolet-irradiated DNA is a photoproduct other than pyrimidine dimers. Such a photoproduct can also be induced by irradiation of the DNA in vivo, i.e. within the cells.

Results of projects A.2.2 and A.2.3

Project leader : Dr. P. van de Putte
Research workers: Dr. B.W. Glickman
Drs. H.L. Heyneker
Dr. C.A. van Sluis
Dr. B. van Dorp (contract ended September 1974)

Title of project: Identification of gene-products determining radiation-sensitivity of bacteria.

Progress report

DNA polymerase I is a protein with multiple enzymatic functions which makes enzyme suitable to catalyze repair reactions. Besides DNA polymerizing activity the enzyme is capable to degraded double stranded DNA in the 5' - 3' direction and single stranded DNA in the 3' - 5' direction. In order to understand the working mechanism of DNA polymerase I *in vivo* in more detail it is helpful to study *E.coli* mutants with a defect in the polA gene (coding for DNA polymerase I) affecting the different functions of the enzyme. It was shown already that the polAI mutation affects the polymerizing and 3' - 5' exonucleolytic activities; on the other hand we have characterized the polA107 mutation which lacks 5' - 3' exonucleolytic activity only. From genetic studies we believe that *E.coli* resA (another mutation affecting DNA polymerase I) is lacking all enzymatic activities. This is in contradiction with the results obtained by Lehman and Chien. We have tried to prepare a specific substrate to measure 5' - 3' exonucleolytic activity: Tritium-labelled DNA from *E.coli* was irradiated with UV light and incubated with UV-specific endonuclease from *M.luteus* resulting in 3'P 5'OH single stranded breaks next to pyrimidine dimers. Subsequently the 5'OH endgroups can be phosphorylated by DNA kinase and γ^{32}P ATP. The material obtained will serve as a specific substrate for 5' - 3' exonuclease: upon incubation with the enzyme the liberation of ^{32}P over ^{3}H into acid-soluble product is a measure for 5' - 3' exonucleolytic activity. Although we have purified UV-specific endonuclease, DNA kinase as well as γ^{32}P ATP of very high specific activity we were untill now unable to label the 5'OH endgroups. Work is in progress to sort this out and to characterize the resA mutant.

Bacteria treated with toluene become permeable to low molecular weight compounds like the direct precursors for DNA replication and repair. The advantage of this semi *in vitro* system is that the organization of the cells is largely intact. It is possible to differentiate between DNA replication (which is ATP dependent) and repair by using *E.coli* mutants temperature sensitive for DNA replication or by using specific drugs. It has been shown that DNase I can penetrate toluene treated bacteria and induce repair-replication. Also when wild-type cells are irradiated with UV light repair is induced. Toluenized cells of *E.coli* uvrA or uvrB mutants (lacking UV-specific endonuclease activity) do not show repair synthesis after UV irradiation, however by adding UV-specific endonuclease purified from *M.luteus* we have found that repair synthesis occurs. So it seems possible to substitute for a genetic defect in repair by adding the missing proteins from the outside and this method opens the possibility to identify unknown gene-products.

E.coli strains carrying the RorA mutation are sensitive to γ-rays, resistent to UV-light, but behave normal in DNA recombination. By genetic techniques it was shown previously, that the rorA mutation is located very close to the recB gene, which codes for an ATP-dependent DNase. The ATP-dependent nuclease was purified from E.coli RorA and the properties compared with the ATP-dependent nuclease prepared from isogenic wild type E.coli cells. It is concluded that the two ATP nucleases are indeed different in their properties with respect to ATP consumption, optimum ATP concentration and ionic strength during DNA breakdown. DNA stabilizes the enzyme considerably, which is an indication that the enzyme forms a complex with DNA.

Since a few years methods are described to amplify genes by means of manipulation of the DNA in vitro. We believe that this system might be a powerful tool to facilitate the identification of unknown gene products especially those which are involved in repair. We have started to set up this technique and therefore we have purified different enzymes involved in genetic engineering like restriction endonuclease EcoR1, λ exonuclease, exonuclease III and DNA ligase. Terminal transferase, which is also an important enzyme for genetic engineering was a generous gift of the group of Dr. F. Campagnari, Euratom centre, Ispra, Italy, Also with advanced genetical techniques it seems possible to amplify genes with the aid of bacteriophage mu-1, and we will make use of this technique as well in the near future.

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Contractor : Department of Radiation Genetics and Chemical Mutagenesis
Contract No. : 102-72-a-1-BIAN
Head of Research Team : Prof.Dr. F.H. Sobels
General subject of Contract : The effects of radiation on genetic and biochemical systems

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The major thrust of research with Drosophila melanogaster has involved radiation induced chromosomal mis-segregation and alteration, as well as the modification of induced changes especially with respect to the maternal repair system.

Various lines of investigation were continued on the effects of modifying the physiological environment and the genotype of the maternal oocytes on the recovery of genetic changes from irradiated male germ cells. The effect of feeding caffeine to females mated to males whose spermatozoa had been irradiated was studied in experiments which utilized two kinds of bithorax mutants whereby genetic damage was measured as transvection type rearrangements. The results tend to support the contention that 1) caffeine inhibits repair (and misrepair) and 2) one of the bithorax strains has a defective repair mechanism.

In contrast to the modifying effect of caffeine observed above, the yield of translocations produced by irradiating pupal spermatids, was unaffected by crossing the males to caffeine treated females. Since breaks produced in pupal spermatids remain available for interaction with breaks produced in spermatozoa, the present results suggest that during the time between the irradiation and hatching a change occurs in the nature of the breaks which makes them incapable of responding to caffeine in the oocyte.

The metabolic inhibitor, NaF, which had been shown to significantly modify radiation and chemically induced genetic damage when applied as a pre-treatment to adult males, was found to be ineffective as a pre-treatment to females in significantly altering the recovery of X-ray rearrangements induced in the paternal genome. Extensive experiments involving various doses have failed to confirm the previously noted significant increase in translocation frequency among paternally derived chromosomes when treated (3,000 R) males were mated to females which had received a small (20 R) treatment. On the other hand, more data have been obtained to indicate that similar treatments produce a significant decrease in dominant lethal frequencies.

Further studies of induced non-disjunction have provided two additional lines of evidence to support Parker's concept that much of radiation induced non-disjunction is a consequence of interchange between

non-homologous chromosomes. On the other hand, cold-ageing induced non-disjunction appears to be a completely different phenomenon.

Research is continuing on the analysis of marked radiation sensitivity changes in female germ cells and the role of mutator genes on induced mutability in females.

As part of the studies on the radiation induction of chromosomal aberrations in somatic and germ cells of the mouse and monkey, in vitro irradiation of rhesus monkey blood yielded evidence that does not fit the concept of Brewen and Preston that the induction of dicentrics in peripheral blood lymphocytes of different mammalian species is proportional to the "effective" chromosome arm number. Furthermore, the observed ratio between reciprocal translocations and dicentrics varied with the exposure level. This result differed from the equality of induction reported for plant material.

The expression time for radiation induced mutations and dose-response relationships have been examined for two in vitro systems, mouse L5178Y cells and human skin fibroblasts. For the mouse material, there was no indication of a relationship between expression time and dose as had been reported with Chinese hamster cells. However, in contrast to the human skin fibroblasts where full expression occurs within 3 days and a stable frequency is observed over a period of at least 14 days, in the mouse cells optimal expression is only reached 6 to 7 days after irradiation and then decreases from the 7th to the 11th day.

Studies on the nature of the 8-azaguanine resistant mutants obtained in the above work as well as with BSC cells are continuing.

In a search for evidence of storage effects for chemically induced genetic damage in mammalian cells, in vitro treatment of human foreskin fibroblasts with TEB and of xeroderma pigmentosum cells with AAF was found to induce significant increases in chromosomal aberrations. However, there was no consistent evidence for an increase in the frequency of chromosomal damage with increasing storage time.

Project No. : I.1

Head of Project and scientific staff : Prof.Dr. F.H. Sobels
D. Mendelson

Title of Project : Effects of the physiological environment and the genotype of the maternal oocytes on the recovery of translocations from irradiated male germ cells

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The effect of treatment of females with two metabolic inhibitors, caffeine and NaF, on the recovery of genetic damage induced by irradiation in spermatozoa, was studied.

A. Caffeine experiments. The following reciprocal crosses were performed: (1) ♀♀Ubx e^4/Payne ca X bx^{34e} ♂♂, (2) ♀♀ bx^{34e} X Ubx e^4/Payne ca ♂♂. The females were fed for 3 days with a solution of 0.2% caffeine + 10% honey in water and then mated to males that had been irradiated with 2000 R of X-rays. The rate of rearrangements in the 3rd chromosome, induced by X-ray in the paternal genome was measured by scoring for transvections type mutants. When Ubx females were used, caffeine had no effect on the already low rate of rearrangements. When bx females were used, the rate of rearrangements was higher and caffeine treatment caused a small but significant reduction in the frequency of rearrangements.

These findings support the assumptions based on previous findings that:

(1) Caffeine inhibits repair (and misrepair) mechanisms in Drosophila oocytes;

(2) Ubx females probably have a defective repair mechanism.

B. NaF experiments. 3 day old Inscy; bw; st p^p females were fed for 18 h. with a solution of 0.025% NaF + 10% honey in water, and mated to ring X males, R(1)2, yB/B^sYy^+, that had been irradiated with 2000 R of X-rays. The effect of NaF on the recovery of sex chromosome loss and autosomal translocations induced in the paternal genome was studied. The results show that in contrast to caffeine, treatment of females with NaF does not produce any consistent and significant alteration in the frequency of sex chromosome loss or translocations recovered from irradiated males. With respect to the latter end point of damage however, there is a tendency for the frequencies to be slightly lower in the

NaF series, but the difference does not reach statistical significance.

The present results concerning NaF do not support the expectation that NaF might act as an inhibitor of maternal repair in Drosophila oocytes.

Project No.:I.1.2

Head of Project and scientific staff : Dr. K. Sankaranarayanan

Title of Project : Effects of small doses of radiation to Drosophila females on the recovery of sex-linked lethals, autosomal translocations and dominant lethals from mature spermatozoa sampled from irradiated males.

The preliminary results of this study were reported in the 1973 Annual Report. 7-day-old Oregon-K males were irradiated with 3000 R and mated to females that had either been irradiated with 20 R (group MF) or not at all. The results showed that (i) the frequencies of sex-linked recessive lethals were essentially the same in both groups and (ii) the frequencies of II-III translocations were higher in the MF than in the M group. The tentative hypothesis suggested to explain the above observation was that small doses of radiation presumably affect some metabolic process which facilitates both repair and misrepair of induced chromosome breaks. As a consequence, some of the chromosome breaks which would otherwise have been eliminated as dominant lethals contribute to the formation of translocations. In fact, in a study of the induction of dominant lethals under similar experimental conditions, the frequencies were significantly lower in the MF than in the M group.

In further attempts at extending the observations to other exposures (males) and collecting more data, it turned out that the results obtained at 3000 R could not be confirmed. The pooled data of the different experiments carried out at different exposures are given below:

Sex-linked lethals

		Group-MF			Group-M		
Exposure to ♂♂	No.of Expts.	No.Chr. tested	No.lethals or Transl.	Freq. (%)	No.Chr. tested	No.lethals or Transl.	Freq. (%)
2000 R	3	6169	502	8.1	5674	404	7.1
2500 R	5	7592	758	10.0	7771	709	9.1
3000 R	10	9072	1108	12.2	8882	1048	11.8
4000 R	4	2410	319	13.2	2267	323	14.2
		Translocations					
2000 R	3	4629	273	5.9	4248	287	6.8
2500 R	5	5356	489	9.1	5760	463	8.0
3000 R	10	5517	629	11.4	6081	656	10.8
4000 R	4	1521	201	13.2	1361	182	13.4

The reasons for these unexpected results are not clear. It thus appears that the hypothesis tested is untenable. On the other hand the frequencies of dominant lethals were lower in the MF than in the M group in all three experiments conducted thus far at 3000 R (MF 69.5% versus M 75.5% over 10,000 eggs in each group) and one experiment at 2000 R (MF 55.2% versus M 59.2%; over 2500 eggs in each group).

Project No. : I.2

Head of Project and scientific staff : Prof.Dr. F.H. Sobels

Title of Project : Caffeine treatment of the maternal repair system and repair of chromosome breaks induced in Drosophila spermatids

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Earlier experiments by Sobels and co-workers showed that after irradiation of Drosophila spermatids in N_2, post-treatment with O_2 leads to a reduction in frequency of various categories of genetic damage, in comparison to that observed after N_2 post-treatment. More recent evidence for the operation of repair processes in the female oocytes raised the question whether the different effects of the above post-treatments perhaps do not act directly on the spermatids, but rather affect differentially the susceptibility of the induced chromosome breaks to the female repair system. To test this idea experiments were undertaken to determine whether: (1) the yield of translocations from males irradiated as 36-hour old pupae can be modified by mating these to females that had received caffeine treatment, and (2) the inhibition of maternal repair by caffeine would have any effect on the recovery of translocations from males irradiated as 36-hour old pupae in N2 and post-treated with N_2 or O_2. The results show that treatment of the females with caffeine does not modify the yield of translocations obtained from irradiated 36-hour old pupae, and this is so, irrespective of whether irradiation was given in air or N_2, followed by N_2 or O_2 post-treatment. These results thus are different from those obtained when irradiated adult males are mated to caffeine-treated females, as in the latter case caffeine, by inhibiting misrepair, results in a reduction of the translocation yield. The present results with pupal spermatids suggest that during the period of 60-70 hours elapsing between irradiation and hatching a change occurs in the nature of the breaks which makes them no longer respond to caffeine in the oocyte. This finding is of interest, since Sobels' results with dose fractionation showed that breaks induced in 36-hour pupae remain available for interaction with breaks produced much later, when the same cells had developed to mature spermatozoa. This apparent discrepancy between the two different sets of data merits further investigation.

Project No. : I.3

Head of Project and scientific staff : Dr. K. Sankaranarayanan

Title of Project : How are the marked sensitivity changes from immature (stage 7) to mature (stage 14) oocytes brought about?

In the 1973 Report, the results of experiments involving irradiation of Drosophila females of different ages on the frequencies of dominant lethals and autosomal recessive lethals were presented. Most of these were carried out at an exposure of 3000 R. It was found that (1) the oocytes sampled from females of age 4, 8 and 12 hrs show similar sensitivity to the induction of dominant lethals; (ii) the sensitivity shows a gradual increase with increasing age of the females up to about 18 hrs after which the increase is steep; by about 28 hrs of age, the frequency of dominant lethals is 1.7 times that observed in the 4-12 hr groups; (iii) from about 28 hrs of age onwards, the increase is more gradual and (iv) in contrast, the frequency of II chromosome recessive lethals in oocytes sampled from 4 hr-old and 24-hr-old females are nearly the same.

In subsequent experiments at 500 R, it has been found that the frequency of II-chromosome recessive lethals increases from 4 to 24 hrs (1.6% versus 2.4%) after which it seems to level off. (2.5% at 30 hrs; 3.0% at 36 hrs and 2.7% at 48 hrs). These experiments are currently being extended to include other age groups.

Project No. : 1.4

Head of Project and scientific staff : Prof. Dr. F. H. Sobels
Drs. A. W. van der Wielen

Title of Project : The effect of several X-ray qualities on the induction of genetic damage in spermatocytes of Drosophila.

Doubt has been cast on the axiom that the frequency of radiation-induced mutations does not depend on the wave length of the X-irradiation. Haendle (1971) showed that radiation induced mitotic recombination in Drosophila is dependent on the X-ray spectrum and the radiation intensity. To test the effect of X-ray quality (50 and 100 keV) on the induction of genetic damage in spermatocytes of Drosophila, special stocks are being built which make it possible to compare several different kinds of genetic damage in one experiment.

The stocks are the following:

male stock: y w^a f/B^s Y y^+; dp b cn bw/ +

female stock: In(1)sc^{S1L} sc^{8R}+ dl-49, y; dp b cn bw ; e

These stocks make it possible to score induced crossing over in the second chromosome, translocations, recessive sex-linked lethals, complete and partial sex-chromosome loss.

Because of the necessity of learning to use accurate physical radiation techniques with high precision dosimetry, a visit was paid to Prof. Dr. Becker of the Genetics Department of the University of München and to the Institute for Radiation Protection of the Society of Radiation Research in München. There were discussions on the physical problems of the radiation technique.

The X-radiation will be given with a special tube with Beryllium window (Superficial radiation therapeutic apparatus, RT-100, Philips-Müller). Two spectra will be used, namely 50 keV, 0.78 mm Al, and 100 keV, 1.7 mm Al. The X-ray spectra of both radiation qualities will be measured in cooperation with the Institute for Radiation Protection in München.

The mitotic recombination technique of Dr. J. Haendle was also been learned. This technique will be used to compare the results of the genetic experiments with the physical data of X-radiation.

Project No. : II.1
Head of Project and scientific staff : Dr. B. Leigh
Title of Project : X-ray induction of autosomal non-disjunction and half-translocations in oocytes

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A. From work in previous years, it was known that radiation induced mis-segregation of X and C(2) chromosomes are related phenomena. The underlying mechanism was initially tested by irradiating females of the genetic constitution $\underline{Dp(1:1)sc^{v1},y^{2}\ sc\ v\ f\ .\ y^{+}\ ;\ C(2L)RM,b\ pr;\ C(2R),\ px}$. They were then mated to $\underline{y\ w^{a}\ /B^{S}.Y\ ;\ C(2L)RM,\ j\ ;\ C(2R)RM,\ +}$ males. It was expected that gain and loss of the C(2)'s would be accompanied by $\underline{y^{+}}$ alone or X chromosomes which had lost this $\underline{y^{+}}$ marker. In practice, the progeny were difficult to score and the first results were inconclusive.

B. The "quasibivalent" hypothesis, of non-disjunction (Parker) mediated chromatid exchanges between non-homologous chromosomes, predicts that specific types of exchange product can be recovered as complements of the non-disjunctional products. Together with Sobels and Parker, a scheme was designed to recover detachments of C(2R)RM chromosomes induced by irradiation of immature oocytes. Quasibivalents formed by exchanges between C(2R) and X or 4-th. chromosomes may result in either non-disjunctional gametes or gametes which contain free 2R's. Constructing the necessary stocks was a long and difficult process. More than 6 sets of irradiations have now been carried out. The results show that free 2R's can be induced. Full analysis was not possible because most of the male exceptions were sterile and the marker combinations proved unsuitable for analysis in females. A modified scheme has now been designed and is being constructed.

C. Cold-ageing of mature oocytes is known to induce non-disjunction. This appears to be a completely different phenomenon from spontaneous and radiation induced non-disjunction. Marked X and C(2) chromosomes are now being used to study this type of non-disjunction and to compare the spectrum of exceptions induced by cold-ageing mature oocytes and irradiation of immature oocytes.

Project No. : II.2
Head of Project and scientific staff : Dr. B. Leigh
Title of Project : Are the types of damage induced by radiation in spermatocytes similar to those induced in oocytes?

A) The study on C(2R)RM detachment has been postponed because of difficulty in constructing the required stocks.

B) Strangio (D.I.S. 45 : 132) recovered attached X-Y chromosomes when he irradiated the spermatocytes of ring-X males. Such chromosomes could result either from complex exchanges involving at least three breaks or from a two break exchange giving a centromere fusion or dicentric. Experiments were initiated firstly to confirm Strangio's observation and secondly to analyze the recovered chromosomes.

At the same time a comparison is being made between the exchange products recovered from irradiated spermatocytes with ring-X and rod-X chromosomes.

From the initial exploratory irradiations it has already been confirmed that attached X-Y chromosomes can be induced. On simple testing these can already be classified according to viability and fertility with or without different Y chromosomes. This indicates that complex exchanges and loss of the centric region of one of the chromosomes is the probable mechanism of formation. A dose response curve is now being constructed with doses of OR, 200 R, 400 R, and 500 R. These are relatively low doses for Drosophila males, but spermatocytes are very sensitive.

Project No. : II.3

Head of Project and scientific staff : Prof.Dr. F.H. Sobels

Title of Project : Studies on the relation between radiation-induced chromosome exchange and non-disjunction

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Parker and coworkers have developed a concept that interchange plays a causative part in the production of non-disjunction by X-irradiation in immature (stage-7) oocytes. In his studies Parker followed exchange between X and Y or between X and 4^{th} chromosomes and then studied aberrant segregations of X and 4^{th} chromosomes. The underlying idea is that when non-homologous chromosomes, by exchange following breakage, are tied together in a "quasi-bivalent", then homologues of the chromosomes in question segregate independently and thus may lead to hypo- or hyperploid female gametes.

To test whether the same principle would be valid for non-disjunction of compound second chromosomes, Sobels carried out a series of dose-fractionation experiments. The results showed, in brief, that although there is a significant reduction in the frequency of non-disjunction after fractionation of a 3000 R exposure (in two 1500 R exposures separated by an interval of 3 hours), this is not at all observed after splitting exposures of 2000 and 1000 R. For a further investigation, fractionation of an exposure of 2000 R into two 1000 R exposures (with 3 hrs between fractions)was studied with stocks which permit the simultaneous recovery of both exchanges between compound (attached)X-chromosomes and the 4^{th} chromosome, and non-disjunction of the X- and 4^{th} chromosomes. The results of a large numer of replica experiments convincingly demonstrate that both chromosome exchanges and the associated non-disjunctional phenomena are significantly reduced in frequency by exposure fractionation; the data obtained with this system thus provide evidence in favour of Parker's concept.

Project No. : III

Head of Project and scientific staff : Dr. A.P. Schalet

Title of Project : Quantitative and qualitative characterization of radiation induced heterochromatic rearrangements

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The study on X-heterochromatin breakpoints has been temporarily delayed. However, some of the stocks designed for that project have proved to be useful for a refined analysis of Y-chromosome rearrangements. R(1)2, y $B/B^{S}Yy^{+}$ males are widely used for experiments in which scoring for partial and complete sex-chromosome loss provides a convenient end point to measure induced genetic damage in the F_1 generation. It is of considerable interest to identify the genetic events responsible for the loss of the y^{+} and/or B^{S} marker. As a contribution to such an analysis a scheme has been used whereby many exceptional offspring lacking both of the distal tip Y-chromosome markers may be identified in the F_1 as partial losses of the Y chromosome. This method takes advantage of the fact that the essential bb locus present close to the centromere of the Y-chromosome is absent in the ring-X chromosome. Two crosses were used in which males were irradiated with 3,000 R and crossed to females heterozygous for a bb locus deficiency:

R(1)2, y $B/B^{S}Yy^{+}$ males X (I) y sc^{4} su(f) w^{a} sc^{8},(bb^{-})/y ac In49 v f mal^{1} su(f)

or

(II) C(1)RA, y ac v f mal^{1} su(f), $(bb^{-})/y^{+}Ymal^{106}$

In cross (I) all F_1 males receiving the bb deficient X from their mother and exhibiting marker loss, must represent partial Y losses, whereas males receiving the balancer X chromosome from their mother and exhibiting marker loss, may represent partial or complete loss of the paternal X or Y. In cross (II) all F_1 compound-X females which show marker loss must represent partial Y losses.

Some major features of the data obtained thus far:

Cross (I) : 1. Induced partial Y losses among males carrying the bb^{-} chromosome, 2.1% (Losses of: $y^{+}B^{S}$-30; y^{+}-19; B^{S}-26)

2. Induced partial and complete losses of X or Y, 4.4%. (These include Y chromosome derived losses of: y^{+}- 9; B^{S}-30)

(II): 1. Induced partial Y losses, 1.6%. (Losses of: $y^{+}B^{S}$-25; y^{+}-6; B^{S}-27)

In accordance with previously reported work of others, single marker losses of B^{S} are more frequent than single marker losses of y^{+}. However, it can be seen that the losses of y^{+} and B^{S} are about as frequent as

losses of B^S alone, 55 vs. 53, among those cases in which the simultaneous loss of y^+ and B^S could be identified as involving partial Y chromosome losses. From the analysis of fertile F_1 individuals, including more than 40 in which the B^S marker alone appeared to have been lost, it is already clear that numerous cases do not represent simple deletions, but involve more complex rearrangements including types of position-effect translocation which have been sporadically reported in the literature.

In addition to the data given above for the frequency of losses which appeared as exceptional F_1 flies expressing a non-mosaic deviant phenotype, a number of mosaically expressed phenotypes involving the y^+ and/or B^S markers were observed and the fertile cases are being analyzed along the lines indicated above.

Project No. : IV

Head of Project and scientific staff : Dr. K. Sankaranarayanan

Title of Project : The role of mutator genes on radiation-induced mutability in female germ cells of Drosophila

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The stocks containing the mutator genes were made available to us through the courtesy of Professor M.M. Green. Using these, new stocks were constructed which were similar but for the absence of the mutator genes. The response of stage 7 oocytes to X-rays at exposure levels of 750, 1500 and 3000 R was studied in experiments which measured X-chromosome losses. The results available thus far seem to show that the mutator stock may be more sensitive. The corrected frequencies of X-losses are: 0.10% (750 R), 0.44% (1500 R) and 2.64% (3000 R) in the mutator females; the corresponding frequencies in the comparable control stock are 0.07%, 0.32% and 1.95%. Experiments are continuing to expand the data at each of these exposures. In addition, experiments on recessive sex-linked lethals in these two stocks have been started.

Project No. : V

Head of Project and scientific staff : Dr. A.D. Tates

Title of Project : Are there storage effects for chemically induced genetic damage in mammalian cells?

In 1973, normal diploid human foreskin fibroblasts in stationary phase were treated with various concentrations of the tetra-functional alkylating agent tetra-ethylene-imino-benzoquinone (TEB). The cells were washed and then stored in the stationary phase for 0 hr or one week. At the end of each of these periods the cells were stimulated to divide and chromosome preparations were made 70 hrs later. The data obtained showed no difference in the frequency of chromosome aberrations in the stored and unstored cells. In fact the frequencies did not differ markedly from control values. To explain the unexpectedly low aberration frequencies it was postulated that the chromosome preparations were made when a substantial fraction of the cells were undergoing their second mitotic division after stimulation of cell division. Under these circumstances it was likely that various types of induced aberrations present during the first mitotic division were eliminated by the time the cells were in the second mitotic division.

In 1974, this idea was tested by reducing the growth period from 70 hrs to 38 hrs. Pilot experiments had shown that the selection of a 38 hr period guaranteed that the chromosome preparations are only obtained from cells in the first mitotis after growth initiation. The 38 hr-growth period was applied in two large storage experiments (4600 cells scored) in which cells treated with two concentrations of TEB (1.8 and $3.6.10^{-3}$ uM) were stored for 0 hr and one, two, or three weeks. Results of these experiments showed that:

a) The frequencies of chromosome aberrations in TEB treated cells were often significantly higher than in untreated cells, especially in unstored cells.
b) There is no good evidence for an increase in the frequency of chromosome aberrations with increasing storage times.
c) The absence of a clear cut storage effect is mainly attributed to cell selection processes operating during storage.

To inquire whether the absence of a storage effect might have been, at least in part, due to the interaction of repair processes and the effect sought, xeroderma pigmentosum cells were used in the most recent storage experiment (1400 cells scored). The chemical used here was N-acetoxy-acetyl-aminofluorene (AAF), one that is known to produce UV like lesions. The

rationale for using xeroderma cells is that they are deficient in excision repair. AAF ($3.6.10^{-3}$ uM) induced significant amounts of chromosome damage in unstored cells and in cells which had been stored for one week. However this experiment also failed to show any consistent storage effect.

Project No. : VI.1

Head of Project and scientific staff : Dr. J.W.I.M. Simons
Drs. A.G.A.C. Knaap
Drs. Y.C.E.M. de Ruijter

Title of Project : Mutation induction in diploid somatic cells in vitro

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The expression time of radiation induced mutations in mouse L5178Y cells has been determined. In contrast to data obtained with Chinese hamster cells, no indication has been found that the expression time is related to radiation dose. The expression time curve itself is difficult to interpret. First of all the optimum expression is reached six to seven days after irradiation, which means about twelve cell generations. This is far too much to be explained solely by the disappearance of normal gene products. Secondly the mutant frequency is not stable after maximum expression but decreases from the seventh to the eleventh day. This cannot be explained by assuming that the mutants are at a selective disadvantage when cultured together with wild type cells as a reconstruction experiment indicated that the mutant cells have a slight advantage under these conditions. Because of these phenomena the dose-response curve of radiation induced mutations is not easy to interpret. Taking into consideration the optimal mutant frequencies only, the dose-response is non-linear and the induced mutant frequency per R increases about linearly from 1.9×10^{-7} to 3.1×10^{-7}/R with increasing exposures (200 - 600 R). The induced mutant frequencies vary little from experiment to experiment.

The expression time for radiation induced mutations in human skin fibroblasts has been determined also. The expression time curve indicates full expression of the mutants within three days and a stable frequency over the time period tested (up to fourteen days). The induced mutant frequency at an exposure of 250 R is 3.8×10^{-7}/R. This figure is the median value from 5 experiments.

Project No. : VI.2

Head of Project and scientific staff : Dr. J.W.I.M. Simons
Drs. A.G.A.C. Knaap
Drs. Y.C.E.M. de Ruijter
Drs. P.C.F.M. Verschure
Ir. A.A. van Zeeland

Title of Project : Analysis of 8-azaguanine resistant mutants

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1) The heat stability and electrophoretic properties of three mutants from human diploid skin fibroblasts was compared with those of wild-type cells. No differences were obtained in the electrophoretic patterns but an increase in heat stability was observed in one mutant, indicating a change in the structural gene for the HGPRT enzyme.
2) Complementation of mutants selected from human diploid skin fibroblasts was studied by cell hybridization of mutants with one another or of mutants with Lesch-Nyhan cells. HGPRT determinations were carried out on the hybridized cells. So far no complementation has been found.
3) The stability of mutants derived from human diploid skin fibroblasts was tested by culturing mutants in the presence or absence of the drug. All five mutants which had no or very little residual HGPRT activity proved to be stable, whereas only two out of nine mutants which had residual enzyme activity were stable.
4) In another experiment mutants were recloned in medium with and without the selective agent. In this way the presence of wild-type cells among the mutant cells could be excluded as a possible interpretation for increase in HGPRT activity. Two of the three mutants which could be tested in this way showed instability. More extensive experiments on re-cloning and progeny testing of mutant clones had been planned with mouse lymphoma cells. As 8-azaguanine could not be used as a selective agent in this cell system, mutants were selected with 6-thioguanine. All the 23 mutants so far tested for their HGPRT activity turned out to be completely enzyme deficient. Thus mouse lymphoma cells appear not to be suited to study this problem. Therefore BSC cells are now being tested in this respect. A first experiment indicated a large heterogeneity in HGPRT activity among clones derived from a single mutant with residual enzyme activity.

Project No. : VI.3

Head of Project and scientific staff : Prof.Dr. F.H. Sobels
Drs. P.P. van Buul

Title of Project : Comparative studies on the induction of chromosome aberrations in somatic cells and spermatogonia of mouse and monkey

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The results obtained in the fractionating experiment on mouse spermatogonia (see 1973 report) were published. The analysis of the correlation between radiation-induced stable chromosome aberrations in spermatogonia, bone-marrow and peripheral blood lymphocytes of the rhesus monkey was finished. At each exposure level (100, 200 and 300 rad), 2 monkeys were studied. Due to the limited capacity of the available radiation machines and the size of mature rhesus monkeys, it was impossible to give total body irradiation. Consequently, the experiment was split into two parts: one group of 6 mature monkeys received local testis irradiation and 6 small immature male monkeys received total body irradiation. Chromosome preparations were made 8 months after the irradiations.

It was demonstrated that reciprocal translocations were induced in stem cell spermatogonia. A total of 4425 cells were analysed including 500 control cells from 3 different animals. The translocation frequencies were low in comparison with results obtained with other species. The dose-effect relationship showed a clearly humped curve with a peak at the 200 rad point. The available data are: 0.2% translocations at 100 rad, 1.6% at 200 rad and 0.3% at 300 rad. In the peripheral blood the results of Weber and Howel were confirmed, that is, the yield of dicentrics in cultured lymphocytes of the rhesus monkey, scored several months after irradiation, is very low. The frequencies of symmetrical translocations were also very low and showed no dose-response curve. Study of the bone marrow preparations revealed that also in haemopoetic stem cells reciprocal translocations can be induced. The frequencies were much lower than expected from mouse experiments, but increased with increasing dose. The dose effect curve was close to that obtained for induced reciprocal translocations after in vitro irradiation of rhesus monkey blood (see below).

To get more information about induced chromosome aberrations in somatic rhesus monkey cells, a dose-effect curve for the induction of dicentrics and reciprocal translocations after *in vitro* irradiation of rhesus monkey blood was constructed. The radiation doses were the same as in the spermatogonial irradiation experiment, i.e. 100, 200 and 300 rad X-rays with an exposure rate of 150 R/min. After *in vitro* culture of the lymphocytes with a modification of the technique of J. Egozcue and M. Vilarasan de Egozcue, 600 cells at each dose level were scored for chromosome aberrations. The results show that:

1) The rhesus monkey data do not fit the findings of Brewen and Preston (Mutation Res., 26 (1974) 297-305) that the induction of dicentrics in peripheral blood lymphocytes of different mammalian species is proportional to the "effective" chromosome arm number. The frequency of dicentrics per unit in the rhesus monkey was significantly lower than expected on the basis of the "effective arm" number. Furthermore, the ratio between aberrations in the peripheral blood lymphocytes and in spermatogonia was 25 : 1 at 100 R, 8 : 1 at 200 R, and 90 : 1 at an exposure dose of 300 R.

2. The observed ratio between reciprocal translocations and dicentrics varied with exposure; this suggests that these types of chromosome aberrations are not always induced with the same relative frequencies. This observation is of considerable interest because it has been reported that the frequencies of reciprocal translocations and dicentrics induced in G_1 chromosomes of *Vicia faba* were equal.

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Laboratory for Physiological Chemistry
Contract No. 102-72-a 1 BIAN
Dr. A.J. van der Eb

STUDIES ON THE MECHANISM OF TRANSFORMATION BY ONCOGENIC DNA VIRUSES

There is considerable evidence suggesting that only a few viral genes are involved in transformation by oncogenic DNA viruses. Identification and characterization of the transforming genes and their products is of great importance for obtaining an understanding of the process of virus-induced transformation, and possibly also for carcinogenesis in general.

In 1974, the investigations on the localization and isolation of the transforming genes of Adenovirus- and SV_{40}-DNA have been continued. Using bacterial restriction endo nucleases, it has been possible to isolate a small specific fragment from Adeno 2 and 5 DNA, which is capable of transforming cells in vitro. Experiments have been started to identify the proteins which are coded for by the viral genome, using cell-free protein synthesizing systems from wheat germs or from mammalian cells.

Similar work has also been carried out with the DNA of SV_{40} virus. It was shown that not only the circular viral DNA but also linear molecules and possibly fragments of the DNA are capable of causing transformation.

Experiments have been started to isolate transforming fragments from DNA of highly oncogenic Adenovirus 12 and of weakly oncogenic Adenovirus 7. (Adenoviruses 2 and 5 are non-oncogenic viruses; all types of Adenoviruses, however, are capable of transforming cells in vitro).

The purpose of these investigations is to compare the transforming genes and the gene products from the various Adenovirus subgroups and from SV_{40}, and to explain the differences in biological behaviour between oncogenic and non-oncogenic Adenoviruses.

Project No. : 1

Research workers: Dr. F.L. Graham, Dr. S.O. Warnaar, Drs. P.J. Abrahams, Dr. J. Lupker, Dr. A.J. van der Eb. Cooperation with Dr. C. Mulder, Cold Spring Harbor and Professor W. Fiers, Gent.

Title : ISOLATION OF TRANSFORMING FRAGMENTS OF ADENOVIRUS AND SV_{40} DNA.

Adenoviruses. Previous work has demonstrated that the transforming potential of Adenovirus 5 DNA remained unaffected when the DNA was sheared to half or quarter molecules. Further experiments have shown that the transforming activity remained constant down to a molecular weight of $\pm$ 2 x 10^6 daltons, and disappeared below $\pm$ 1.0 x 10^6 daltons. This result indicated that the transforming DNA segment is not greater than approximately 1.5 x 10^6 daltons (or 5% of the genome).

In order to localize the transforming activity on the Adenovirus 5 genome the following experiments were carried out: (1) DNA was broken into equal halves, and the GC-rich and AT-rich halves were separated and tested for transforming activity. Only the GC-rich (left) half of the genome contained transforming activity. (2) DNA was sequentially degraded from the ends with exonuclease III, followed by incubation with single-strand specific S1 endonuclease. It was found that the transforming activity disappeared when $\pm$ 2% of the DNA was degraded, or $\pm$ 1% of each end. It was concluded from (1) and (2) that the transforming activity is located close to the left-hand end, and begins at a distance of 1 - 1½% from the left end.

Attempts were then made to isolate a specific and small DNA fragment with transforming ability, using bacterial restriction enzymes. Adeno 2 and 5 were cleaved with endo R.EcoRI into 6 and 3 fragments respectively. It was found for both viral DNA's that only the (left terminal) A-fragment contained transforming activity. Since the A-fragments represent more than 50% of the genome, it was necessary to cleave the left half of the DNA further into smaller fragments. Of the several restriction enzymes which were then tested, positive results were only obtained with the endonuclease R-HsuI. For Adeno 2 DNA it was found that transforming activity was associated with the left-terminal Hsu I-G fragment (1.6 x 10^6 daltons, or $\pm$ 7% of the genome), and it was likely that also for Adeno 5 DNA the left-terminal G-fragment was the active one. (For Adeno 5 DNA sofar only a mixture of F + G fragments was tested, since these are very similar in size and

hence could not be separated. The F-fragment is localized in the right half of the molecule). (The cleavage maps of Adeno 2 and 5 DNA with endo R.Hsu I were provided by the Cold Spring Harbor Laboratory, Cold Spring Harbor, N.Y.).

In summary, it was found that a small and specific fragment of Adeno 2 and 5 DNA, which is localized at the left end of the genome and which represents ± 7% of it, contains information for the phenotypical expression, and possibly also for stable perpetuation, of transformation.

Preliminary experiments suggest that also a specific fragment of Adeno 12 DNA (a tumorigenic Adenovirus) contains transforming activity.

SV_{40}. By using the calcium technique, it was also possible to obtain *in vitro* transformation with SV_{40} DNA. It was found that not only circular DNA but also linear molecules of genome size contained transforming activity. Preliminary data also indicate that transformation can be obtained with a specific fragment of SV_{40} DNA, prepared with restriction endonucleases. Since the possibility could not be excluded that this fragment was contaminated with molecules of genome size, the experiments will be repeated with extensively purified fragments.

Protein synthesis. In order to identify the protein(s) coded for by the transforming segment of Adeno 2 and 5 DNA, a project was started to translate viral messenger RNA's in cell-free protein synthesizing systems. Using an extract from wheat germs and a crude preparation of RNA from Adenovirus-infected cells (which largely consists of Adenovirus specific mRNA), it was shown that several distinct polypeptides were synthesized *in vitro* which co-migrate in gel-electrophoresis with viral coat proteins. This suggests that the system can be used to translate viral mRNA. Other systems derived from mammalian cells will also be used, in addition to the wheat germ system. The next step will be to isolate the messenger RNA coded for by the transforming DNA segment, and to use this RNA for *in vitro* protein synthesis.

Laboratory for Physiological Chemistry
Contract No. 102-72-a 1 BIAN
Dr. A.J. van der Eb

STUDIES ON THE MECHANISM OF REPAIR OF RADIATION DAMAGE IN MAMMALIAN CELLS.

Studies on the mechanism of repair of radiation damage in mammalian cells are hampered by the complexity of the cell and in particular by the large size and heterogeneity of the cellular DNA.

Earlier work had indicated, however, that the repair mechanisms in mammalian cells not only act on their cellular DNA, but also on heterologous DNA, such as the DNA of a virus, which has been introduced into the cell.

This observation has opened the possibility of studying the repair processes by using small, well-characterized DNA's from viruses which replicate in the cells.

In this investigation, use is made of the DNA virus, SV_{40}, which contains a circular, double-stranded genome with a molecular weight of 3.5×10^6 daltons, and which can replicate in monkey cells as well as in human cells.

The purpose of this work is to obtain a better understanding of the repair processes of radiation damage in mammalian cells, in particular in human cells, and to study the defects of the repair processes in cells from patients with an increased sensitivity to radiation.

Project No. : 2
Research workers: Drs. P.J. Abrahams, Dr. A.J. van der Eb
Title : STUDIES ON THE MECHANISM OF REPAIR OF RADIATION DAMAGE IN MAMMALIAN CELLS

In 1974 the first phase of this project, which consisted of a study of the UV-sensitivity of SV_{40} DNA in normal and radiation-sensitive cells, has been finished (Manuscript in preparation).

The results can be summarized as follows:
The infectivity of UV-irradiated SV_{40} DNA was studied in normal monkey and human cells. It was found that the survival of double-stranded SV_{40} DNA is considerable worse in cells from Xeroderma pigmentosum (XP) patients, compared to cells from normal individuals. The difference in D_{37} varies between 2 and 7, depending on the XP mutant used. As expected, the survival in a heterozygons XP-strain is similar to the survival in normal cells.

Surprisingly, cells from Porokeratoris and Progeria patients appeared to be capable of repairing UV-irradiated SV_{40} DNA more efficiently than normal cells, at least at low UV doses. This effect will be investigated in more detail.

In order to compare the repair of UV-damage in double-stranded and in single-stranded DNA, pure single-stranded SV_{40} DNA molecules were isolated, using hydroxy apatite chromatography of denatured SV_{40} DNA, after annealing with in vitro synthesized RNA, complementary to one of the SV_{40} DNA strands.

As expected, single-stranded SV_{40} DNA was found to be much more sensitive to UV-irradiation than double-stranded DNA: for the single-stranded DNA a D37 of 130 erg/mm^2 was found in normal cells, compared to 7.25×10^3 erg/mm^2 for double-stranded DNA in the same cells. A D37 of approximately 130 erg/mm^2 has also been reported for Kilham rat virus, as well as for ØX 174 phage, which both contain a single-stranded genome.

The results indicate that the UV-sensitivity of single-stranded DNA in normal cells is considerably greater than the UV-sensitivity of double-stranded DNA in XP-cells, even in cells from the most severe Xeroderma pigmentosum cases, suggesting that Xeroderma cells are capable of repairing some of the UV damage in double-stranded DNA.

List of publications

1. F.L. Graham, P.J. Abrahams, C. Mulder, H.L. Heijneker, S.O. Warnaar, F.A.J. de Vries, W. Fiers and A.J. van der Eb

Studies on in vitro transformation by DNA and DNA fragments of human adenoviruses and simian virus 40.

Cold Spring Harbor Symp. Quant. Biol. 39 (1974).

2. F.L. Graham, A.J. van der Eb and H.L. Heijneker

Size and location of the transforming region in human adenovirus type 5 DNA.

Nature 251, 687 (1974).

3. P.J. Abrahams and A.J. van der Eb

In vitro transformation of rat and mouse cells by DNA from simian virus 40.

Submitted for publication.

4. A.J. van der Eb, C. Mulder, F.L. Graham and F.A.J. de Vries

Transformation of rat kidney cells with specific fragments of adenovirus 2 and 5 DNA.

In preparation.

Contractant de la Commission :

N° du contrat : 099-72-1 BIAB

Chef des groupes de recherche : Jean BRACHET

Thème général du contrat : Effets des radiations sur la stabilité de l'information génétique

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Description générale succincte des travaux réalisés :

The main results obtained were the following in 1974 :

1. Computer analysis of ESR spectra of γ_0 irradiated dAMP at 77° K and during step by step annealing leads to a model of reaction in which adenine anions and cations react with H and OH radicals. NMR studies of frozen DNA solution and of synthetic nucleotides have lead to the suggestion that there are 2 types of bound water structures one of which is more radiosensitive.

2. Proflavine protects λ phage against direct and indirect effects of radiation.

3. Both radiation induced repair (SOS repair) and mutagenesis are correlated with an induced stimulation of DNA replication ; the enzymes involved are under investigation.
A tRNA controlled endo deoxyribonuclease has been identified and partially purified from yeast mitochondria.

4. With the aid of the mutator Mu bacteriophage efficient transposition of chromosomal segments on a F factor have been obtained. New data concerning the replication of λ phage are reported.

5. Regulation networks of λ have been formalized and models have been confirmed experimentally ; the positive and negative controls involved in lysogenisation and lysis were further studied.

6. A new method for isolating a SV40 transcriptional complex in mammalian cells has been developed. The control of genetic expression of hepatoma cells has been studied after their fusion with fibroblasts which inhibits the expression of 4 proteins normally synthesized by the hepatoma cells.

7. Somatic recombination has been studied in mammalian DNA by biochemical and cytochemical techniques and a possible relationship with sisterchromatid exchanges was found.

8. The sensitivity of synkarions of mammalian somatic cells to X rays has been found identical to the sensitivity of the parent cells.

9. The rRNA synthesis in young morulae from mouse embryos is completely inhibited by 500 rads of X rays. Lower doses interfere with the processing of ribosomal RNA. X rays also inhibit the uptake of uridine into DNA at the early morulastage.

10. B lymphocytes bearing surface immunoglobulin are specially sensitive to X rays whereas T lymphocytes appear much more resistant ; it is suggested that some membrane function, important in lymphocyte cooperation, is inhibited. Differentiation of the B cell line in the mouse foetus and the mechanism of antigen binding to immunoglobulin receptor are under study.

11. Labelling experiments suggest that plasma cells receive material from some specific cooperating cell.

12. Irradiated rabbits grafted with allogenic lymphoid cells have been further studied and it was confirmed that the host synthesises antibodies with both recipient allotypic and donor idiotypic specificities.

Résultats du projet n° I.

Chef du projet et collaborateurs scientifiques:

A.J. Bertinchamps; S. Gregoli, R. Mathur-De Vré, P. Moretti, M. Olast.

Titre du projet: Primary effects of radiation on Nucleic Ac

ESR Investigations. (A.J. Bertinchamps; S. Gregoli, P. Moretti, M. Olast)

The possibility of direct computer analysis of ESR data arising from the γ-irradiation of polycrystalline samples was already mentioned in the Euratom Report of 1973. Improvements in the method and clear-cut results since obtained allow us to now describe more thoroughly this technique.

The field of application. γ-irradiation of a biological substance usually gives rise to the concomitant formation of several different radical species. The resulting ESR spectrum, built up by the weighted superimposition of different elementary patterns, cannot be interpreted directly. On the other hand, changing some experimental conditions can alter the relative yields of the various radical species involved. This is revealed by changes in the relative intensities of the lines building up the overall spectrum. Once a series of experimental spectra has been obtained in this way, the computer technique (see below) can be applied with a view to isolating the elementary components of the spectra. This is in fact a fundamental step in the identification of the radical species produced in the irradiated sample.

We are applying this technique to the study of radical formation in various DNA derivatives, γ-irradiated at 77°K in frozen aqueous solution. The frozen state is particularly suitable for the application of this method, since changes in the radical composition can easily be obtained by warming up the irradiated samples.

The technique. All the experimental composite spectra, obtained by heat treating the sample irradiated at 77°K, are first digitalised at 1000 equidistant points and stored on magnetic tape. If we call these spectra S, S', S'',....., and their constituent patterns $s_1, s_2, \ldots\ldots, s_n$, we can then write:

$$S = (w_1)\, s_1 + (w_2)\, s_2 + \ldots\ldots + (w_n)\, s_n$$
$$S' = (w'_1)\, s_1 + (w'_2)\, s_2 + \ldots\ldots + (w'_n)\, s_n$$
$$S'' = (w''_1)\, s_1 + (w''_2)\, s_2 + \ldots\ldots + (w''_n)\, s_n$$

.....

where the coefficients w_i, w_i', w_i'',..... represent the different relative weights of the same s_i component in the S, S', S",..... spectra. It follows that for each pair of experimental spectra there exists one linear combination capable of rendering null the s_1 component, a second the s_2 component and an n^{th} the s_n component. These computer produced combinations will give rise to new spectra, lacking one of the n components and therefore having lesser complexity. Further computer analysis of these combination spectra can in some cases lead to a complete breakdown of the original spectra into their component patterns.

The results. Deoxyadenosine-5'-monophosphate (dAMP) was irradiated at 77°K in frozen aqueous solution, and then annealed, step by step, until disappearance of any detectable ESR signal. Computer analysis of the qualitative and quantitative transformations undergone by ESR spectra during this process enabled us to postulate the following mechanism, accounting for radical reactions involving primary and secondary species and to delineate the corresponding pathways. At 77°K the free radical population formed on dAMP by the direct effect of radiation is mainly composed of adenine anions and cations. Under heat treatment both such adenine ions react with neighbouring water molecules to give respectively H-addition and OH-addition radicals on the adenine base group. Addition of the electron scavenger iodoacetamide to the dAMP solution prior to irradiation inhibits the formation of H-addition radicals selectively, by preventing the formation of their anionic precursors. In addition to the base-located free radicals mentioned, another radical species, located on the deoxyribose moiety of dAMP was also detected. Individual temperature kinetics were described for each radical species.

B. NMR Investigations. (A.J. Bertinchamps; R. Mathur-De Vré)

The effects of γ-irradiation on DNA in aqueous solutions are controlled largely by the mobility and diffusion of water molecules. Previously we described a method using NMR to study the extent of hydration and the mobility of H_2O protons in the hydration layer. We have extended these measurements to investigate in detail the percent hydration and relaxation times at different temperatures for irradiated and non-irradiated frozen solutions of DNA at various concentrations, polynucleotides, and their aggregates (poly (A+U), poly (A+2U), polyAH$^+$). The irradiation was performed in different physical states: liquid solutions at 0°C, frozen solutions at 77°K and irradiating the dry solid before dissolution. Results on the proton resonance spectra of identical solutions in

solvents (50% H_2O + 50% D_2O) and H_2O were also compared to get information on molecular interactions contributing to the relaxation process.

The activation energy values for the proton mobility in DNA solutions suggest the presence of at least two types of bound water structures and the relatively higher sensitivity of one of these to irradiation at 0°C. In general, because of the higly modified mobility and diffusion characteristic of bound water molecules, which are dependent on the structure and the nature of groups present on macromolecules, the "semi-frozen" hydration layer can significantly influence the final radiation damage to DNA mediated through water.

Publications.

S. Gregoli, M. Olast and A.J. Bertinchamps: "Free radicals in γ-irradiated frozen solutions of deoxyadenosine-5'-monophosphate. A computer analysis of the temperature-dependent ESR spectra." Radiat. Res. 60, 388 (1974.)

R. Mathur-De Vré and A.J. Bertinchamps: "An NMR study of the relative interaction abilities of different pyrimidine nucleosides with serotonine". Rad. and Environm. Biophys. 11, 135 (1974).

Communications.

R. Mathur-De Vré and A.J. Bertinchamps: "An NMR study of the hydration of nucleic acids in frozen aqueous solutions" , presented at the VIth International Conference on Magnetic Resonance in Biological Systems. Kantersteg, Switzerland. September 1974.

Résultats du projet n°II

Chef du projet et collaborateurs scientifiques : M. Errera, S. Boiteux, P. Caillet-Fauquet, J. Cornelis, M. Defais, G. Maenhaut-Michel, M. Radman, J. Rommelaere, M. Susskind, G. Villani

Titre du projet : Mechanisms of DNA repair in microorganisms and in mammalian cells

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I. Microroganisms

A. Genetical and biochemical analysis of DNA repair mechanisms and induced mutagenesis : SOS repair and induced mutagenesis (P.Caillet-Fauquet, M. Defais, M. Radman

- Damage to cellular DNA (UV, X rays, chemical mutagens) causes induction of an otherwise silent (repressed) DNA repair system (SOS repair).
- SOS repair is accompanied by induced mutagenesis.
- Damage to phage DNA is a necessary but not sufficient condition for induced mutagenesis ; mutation fixation requires the induction of the bacterial SOS repair system.
- Both, SOS repair and induced mutagenesis are abolished by the recA and lex (exr) mutations.
- SOS repair is efficient for superinfecting phage DNA in a lysogenic bacterium
- Following induction by UV, peak repair and mutagenic activities appear after 30 min., and disappear after about 120 min., of post-irradiation incubation at 37°C
- SOS repair and mutagenesis are totally and irreversibly suppressed by 100 µg/ml chloramphenicol present during at least the first 30 min. of post-irradiation incubation.
- SOS repair is antagonized by cyclic AMP
- Biochemical experiments performed under the conditions of the SOS repair show a replication stimulation to the same extent as is the increase in survival due to SOS repair, suggesting the involvement of an induced error-prone DNA polymerase

B. Identification of enzymes implicated in DNA replication, repair, mutagenesis and recombination (M.Radman, S.Boiteux*, G.Villani*,G. Maenhaut-Michel)

Attempts are being to isolate

- a bacterial endonuclease which preferentially nicks DNA of a λ phage mutant containing a recombinational "hot spot"
- endonucleases from yeast mitochondria
- radiation induced DNA polymerases in E.coli
- to characterize by electron microscopy a complex formed by E.coli endonuclease I, its tRNA cofactor and DNA as the substrate.

*Euratom fellows since 01/10/74 and 01/11/74

C. Protection of phage λ by proflavine (PF) against lethal effects of gamma rays (G. Maenhaut-Michel)

Intercalated PF is an efficient radiation protector against direct effects probably by an electron transfer mechanism efficient along ± ten base pairs ; its presence in the medium efficiently protects the phage against indirect effects.

II. Mammalian cells

A. Somatic recombination and DNA repair in Chinese Hamster cultured cells (J. Rommelaere)

In order to find a possible "multiplicity reactivation" mechanism in mammalian cells, the frequency of recombinational events has been assessed by biochemical methods in somatic cells and correlated with sister chromatid exchanges. The frequency of both processes increases with sublethal doses of U.V. radiation.

B. Pyrimidine dimers detection by a sensitive immunological method (J. Cornelis* and J. Rommelaere)

A sensitive radioautograph method for the detection of intracellular pyrimidine dimers has been developped and is applicable to non lethal doses of U.V.

C. Excision of pyrimidine dimers in Xeroderma Pigmentosum cells partially substituted by BrudR (M. Susskind, J. Cornelis, J. Rommelaere)

When Xeroderma pigmentosum cells are grown in a medium containing a 1/9 mixture of BrudR and thymidine, U.V. irradiation induces both pyrimidine dimers and single strand breaks, which enable 30 p.c. of the dimers to be removed in cells which normally lack excision repair.

Publications

- J. Rommelaere, J. Cornelis, A. Miller-Faurès and M. Errera
The influence of 5-bromodeoxyuridine on DNA repair in Chinese Hamster cells exposed to ultraviolet radiation
Biochim. Biophys. Acta, 340, 388-399 (1974)

- J. George, R. Devoret and M. Radman
Indirect ultraviolet-reactivation of phage λ
Proc. Nat. Acad. Sci. US, 71, 144-147 (1974)

- B.S. Srivastava
The yield of single-strand breaks in the DNA of bacteriophage λ irradiated extra- and intracellularly with gamma-rays in oxic and anoxic conditions
Int. J. Radiat. Biol., 26, 391-394 (1974) - see 1973 report

- J. Rommelaere, A. Faurès-Miller and M. Errera
Isolation of replicating DNA segments from Chinese Hamster cells by density equilibrium centrifugation
J. Mol. Biol., 90, 491-508 (1974)

* Euratom Fellow 1973-74

Résultats du projet nº III

Chef du projet : R. THOMAS and coll.

Titre du projet : The establishment and stability of the state of provirus : genetic factors and effects of physical agents

a) Genetic, biochemical and logic analysis of the control of provirus establishment and maintenance (bacterial cells : C. Dambly, M. Delstanche, L. Desmet, A.M. Gathoye, R. Lathe, J.P. Lecocq, J. Richelle, F. Salomon and R. THOMAS ; mammalian cells : P. Gariglio and S. Mousset)

- Formal study of complex regulatory nets. This study has developped rapidly, in collaboration with the laboratories of logic and numeric systems (Prof. Florine, Dr. Van Ham) and of theoretical physics (Prof. Nicolis). Predictions implicit in current models have been de-crypted by the formal analysis, and experimentally confirmed (in particular, λN^- cro^- lysogenizes efficiently even if it is cII^-, instead of establishing itself as a plasmid). The treatment leading from logic equations to matrices, from matrices to pathways and from pathways to the conditions for following a given pathway, are being automatized, thus permitting analysis of systems of an increasing complexity. The generalization Thomas had to introduce in sequential calculus in order to make it operational in the treatment of genetic control circuits, is now being studied for itself.

- Mechanism of the positive control involved in lysogenization or lysis

Two types of bacterial mutations have been found to relieve phage λ from the need for positive control by the N protein : the "polarity suppressor" mutants (su_A), and a thermosensitive mutant (lycA) with an altered β subunit in its RNA polymerase. These situations and others are further analyzed in order to get a better understanding of transcriptional, and perhaps translational controls.

- Interaction between mammalian cells and oncogenic DNA viruses

A very efficient method for isolating the viral transcriptional complex has been developped by Gariglio. It consists essentially in a treatment of the nuclei of SV40-infected cells with sarkosyl, followed by sedimentation of the chromatin. While the supernatant incorporates virtually no ^3H-uridine if the nuclei derive from uninfected cells, it incorporates very actively if the nuclei are isolated from SV40-infected cells, and this activity hybridizes quantitatively with SV40 DNA. The RNA synthesized by the chromatin does not hybridize detectably with SV40 DNA.

b) Study of the mechanism of integration, excision and induction of the "mutator" bacteriophage Mu (M. Faelen, A. Toussaint)

In addition to the previous results which show that a circular tandem of Mu chromosomes can connect together any two unrelated DNA sequences, it has now been found that mild derepression of prophage Mu leads to transposition, not only of genes adjacent to the prophage, but also of other bacterial genes. One can thus efficiently transpose chromosomal segments on a F factor ; the study of the frequencies of co-transposition of genes should provide a new way in genetic mapping of bacterial genes.

The early gene A seems to be involved in the integration and excision processes.

c) Experimental and theoretical studies on recombination mechanisms (J. De Lafonteyne, M. Couturier, F. Van Vliet and O. Huysmans)

A major finding of J. De Lafonteyne is that, in bacteriophage lambda, the normal process of initiation of replication can be bypassed by a process which involves the viral recombination mechanism (red). "Replication-defective" (O^- and P^-) mutants of λ replicate efficiently (yielding up to 150 phage/bacterium) if a) the cell has been infected by 2 or more phage particles, b) the phage is red^+. Presumably the σ-replication stage is reached through a recombinational event, thus bypassing θ-replication; this can be accounted for in detail in terms of molecular models.

d) Control of genetic expression in mammalian cells (C. and J. Szpirer, A. Resibois and R. Van Geffel)

C. and J. Szpirer are studying the control of the synthesis of serum proteins. The method consists of fusing hepatoma cells which produce each of four proteins (serum albumin, α-foetoprotein, 3rd component of the complement and transferrin) with fibroblasts which produce neither. The results previously reported (systematic extinction of albumin and α-foetoprotein synthesis, maintenance of the synthesis of the two other serum proteins) have been confirmed in several intraspecies crosses. Interspecies hybrids are now under study ; one of the immediate purposes of the work is to allow a comparison with other results (published by M. Weiss and co-workers) and to look for the resistance of possible species-specific effects. Most experiments were performed with a mouse hepatoma cell line isolated in our laboratory (Differentiation, in press).

e) Mutagenic effect of tritiated water in bacteria

This subject has been provisionally abandoned in view of the resignation of N. Wantens.

Publications

- SZPIRER, J. and SZPIRER, C.
  Expression of differentiated functions in mouse hepatoma cells and their somatic cell hybrids
  C.R. conf. internat. "L'alpha-foeto-protein", mars 1974, ed. R. Masseyeff (Nice)

- TOUSSAINT, A. and LECOCQ, J.P.
  Sensitivity of bacteriophage Mu-1 development to rifampicin and streptolydigin
  Molec. gen. Genet. 129, 185-188 (1974)

- SRIVASTAVA, R., TOUSSAINT, C. and LECOCQ, J.P.
  A rifampicin-resistant mutation of E.coli, whose phenotypic expression is dependent on the composition of the medium and the recA allele
  Mutation Res., 23, 25-28 (1974)

- LEFEBVRE, N. and TOUSSAINT, A.
  Induction indirecte du bactériophage tempéré Mu-1
  Arch. Internat. Physiol. Bioch., 82, 188 (1974)

- TOUSSAINT, A. and FAELEN, M.
  The dependence of temperate phage Mu-1 upon replication functions of E.coli K12
  Molec. gen. Genet. 131, 209-214 (1974)

- COUTURIER, M. and VAN VLIET, F.
  Vegetative recombination in bacteriophage Mu-1
  Virology, 60, 1-8 (1974)

- THOMAS, R.
  Essais sur la formulation et le traitement algébrique des raisonnements
  II. Les notions d'existence, d'occurence, de concevabilité
  Automatisme, 19 (n° 11) (1974)

Résultats du projet n° IV.

Chef du projet et collaborateurs scientifiques :
J. Brachet. Collaborateurs : H. Alexandre, Y. Gerin, V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti.

Titre du projet:
1. Radiosensitivity of mouse eggs cultured in vitro during the preimplantation period.
2. Radiation response of somatic cell hybrids.

1. a. RNA synthesis (H. Alexandre, Y. Gerin).

We have found an almost total inhibition of rRNA synthesis in morulae derived from 2 cell-stage eggs which had received 500 R. After irradiation with doses which induce a partial (about 50%) inhibition of cavitation, the transformation of precursor rRNA into 28 and 18S RNA fractions is disturbed. This could result from an abnormal base composition of the rRNA precursor, as proposed by Simić et al. (1969).

b. DNA synthesis regulation. (H. Alexandre, Y. Gerin).

We were able to show that labelled uridine can be incorporated into the DNA of mouse cleaving eggs by : 1) autoradiography : labelling is still found in the nuclei from the 4-cell stage to the blastocyst stage after RNAase treatment of the preparations. The labelling increases when development progresses. In 2-cell-stage eggs, the low level of uridine incorporated is completely removed by RNAase treatment.

2) CsCl gradients centrifugations have shown that radioactivity is associated with fractions which band together with liver nuclear DNA used as a carrier, after incubation of about 300 morulae or blastocysts with uridine ^{3}H during 16 hrs. A period of high sensitivity to hydroxyurea and deoxyadenosine occurs during the third cell cycle of cleavage, indicating the possibility of ribonucleotide reductase activation or synthesis at this stage. In addition, X rays seem to inhibit the metabolic pathway leading to the incorporation of uridine into DNA when they are applied at the 2-cell-stage, but no longer at the late morula stage i.e. after the primary determination, a stage where cavitation is known to be highly radioresistant.

2. Radiation response of somatic cell hybrids. (V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti).

Several synkaryons obtained by fusion of two mutant strains of Chinese hamster fibroblasts have been isolated. The sensitivity to X-rays of parental cells and synkaryons resulting from the fusion of the former, have been compared. To obtain the reciprocal of the terminal slope (Do) and the extrapolation number (n), experimental data obtained from colony counts were fitted to the multi-hit equation of Elkind and Sutton (1960), using a least-square analysis technique. The fitted curves were calculated by minimizing the sum of X-squares weighted by the factor given by Gilbert (1969). These calculations were performed on the CDC 6400 computer at the "Centre de Calcul de l'Université libre de Bruxelles".

Since the Do and n values for hybrid cells are very similar to those of the parental strains, it follows that the synkaryons display the same potential for survival as the parent cells after X-irradiation.

The synkaryons we used arose from parent cells derived from a common ancestry with identical characteristics of radiosensitivity.

Our present purpose is to find out whether the same radiobiological response is obtained in synkaryons deriving from the fusion of mammalian cells of different species.

Publications.

Alexandre, H. Effects of X-irradiation on preimplantation mouse embryos cultured in vitro. J. Reprod. Fert. 36, 417-420.(1974).

Alexandre, H. Effects of X-rays on nucleic acid metabolism during cleavage and gastrulation in Pleurodeles waltlii eggs. J. Embryol. exp. Morph. 32, 147-157. (1974).

Lievens, A., Limbosch, S., Heilporn, V. and Zampetti, F. Morphology and X-ray sensitivity of rat glial cells after cytochalasin B treatment. Arch. Biol. Liège 85, 173, 1974.

Lievens, A., Heilporn, V., Limbosch, S. and Zampetti, F. Survival of synkaryons and the two parental strains following X-ray irradiation Rad. Research 59, 81, 1974.

Limbosch, S., Heilporn, V., Lievens, A., De Coen, J.L. and Zampetti, F. Radiation response of a somatic cell hybrid. Int. J. Radiat. Biol. 26, 197, 1974.

Résultats du projet n° V

Chef du projet et collaborateurs scientiques :

J.Urbain, R.Jeener, G.Urbain-Vansanten, M.Wikler, C. De Vos-Cloetens, A.Van Acker, A.Vienne, N.Tasiaux, C.Bruyns, R.Leuwenkroon, Ch.Wuilmart, H.Balluet, B.Mariame.

Titre du projet : Immunochemical and immunogenetic investigations on the nature and activity of the antibody secreting cells observed in the irradiated animal after transplantation of lymphocytes from an immunized or non immunized donor animal.

- While it is well known that irradiation impairs the immune system, data allowing an interpretation of this effect (J.F.A.P. Miller) are still lacking. Since lymphocytes are heterogeneous with respect to their life span, their differentiation antigen, their function... it is important to compare the radiosensibility of different subsets of lymphocytes. Using immunofluorescence, autoradiography and stimulation with antiimmunoglobulin antisera or phytohemagglutinin, we have compared the radiosensibility of T and B lymphocytes. The results indicate that B lymphocytes, bearing surface immunoglobulin and belonging to a long lived population are especially sensitive to irradiation performed in vivo or in vitro. T lymphocytes which can be activated by PHA are much more radioresistant but the radioresistance is different in vivo or in vitro. A variety of results suggest that a primary effect of radiation is to impair some membrane function important in lymphocyte cooperation. The effects of irradiation are now measured on population of lymphocytes purified by nylon wool columns.

- Previous results from this laboratory (Importance of short lived lymphocytes in immune response ; Immunology, in press) have shown that labelled plasma cells can be labelled in a secondary response if tritiated thymidine is given before antigen boosting. A variety of results suggest strongly that this labelling cannot be due to non specific reutilization of thymidine released from dying cells. Therefore either plasma cells derive from short lived precursors or they receive some labelled material from a specific coo-

perating cell. Elimination of short lived lymphocytes by treatment with vinblastine suggests that transfer of labelled material from a specific cooperating cell effectively occurs. Experiments are underway to understand the physiological meaning of such transfer.

- Experiments using irradiated rabbits grafted with allogeneic lymphoïd cells have been actively pursued. The finding that recipient rabbits synthesize antibodies bearing recipient allotypic markers and donor idiotypic specificities has been confirmed by using homologous antiidiotypic sera raised in allotypically matched rabbits. The specificity of these antiidiotypic sera was checked by numerous methods including immunodiffusion, hemagglutination, radioimmunoassay and immunofluorescence. In addition, it has been shown that crossreactive idiotypes can be found in fractions of the donor antibody, separated by isoelectric focusing. The conclusion has been drawn that crossreactive idiotypes (similar hypervariable regions) can be associated with different framework sequences (subgroup) and that idiotypes are involved in regulatory phenomena. Therefore the synthesis of antibodies in recipient rabbits bearing recipient allotypic markers and donor idiotypic specificities is clearly due to derepression of host B cells, surviving irradiation, able to express idiotypic specificities crossreactive with those of donor antibody. The reasons why irradiated rabbits grafted with allogeneic lymphoïd cells do not suffer from graft virus host disease are being investigated (anti-receptor antibody ?).

- In mouse foetus, lymphoïd stem cells of the B line can be found in liver or in spleen. In the adult such stem cells can be found in the bone marrow. A program has been undertaken to study the appearance of these stem cells and their differentiation into mature B lymphocytes possessing surface immunoglobulins by methods of combined autoradiography and immunofluorescence. It has been shown that B lymphocytes derive from cells proliferating rapidly. When differentiating and acquiring surface immunoglobulins, these cells stop dividing at a fast rate. As soon as surface immunoglobulins appear on the membrane, receptors specific for various antigens can be found. Experiments are underway to characterize differentiation antigens of the stem cell.

- A high frequency of TMV-binding cells has been found in spleen cells from unimmunized mice (about 3 to 4 %). TMV binding is strongly inhibited by previous incubation with antiimmunoglobulin antisera. After stripping of membrane receptors, a full recovery of antigen binding capacity can be observed after 24 hours of culture. Experiments are presented to exclude artefactual fluorescent cells : interaction of TMV with some non immuno-

globulin membrane components, interaction of fluorescent anti-TMV antibody with the Fc receptor of B cells, the binding of TMV to cytophilic immunoglobulins. The occurrence of lymphocytes able to bind several non crossreactive antigens is suggested by three lines of evidence : the high number of antigen binding cells in unimmunized mice, presence of surface immunoglobulins on some TMV binding cells after complete capping of TMV receptors and the direct demonstration of lymphocytes binding TMV and hemocyanin at different membrane sites.

- The mechanism of immunoglobulin receptor binding in the membrane has been investigated by a search for hydrophobic pockets in IgG and IgM. These hydrophobic zones interact with the hydrophobic tail of membrane lipids. The continuation of this program could lead to a better characterization of different receptor types associated with T and B lymphocytes and could allow a clear distinction between these receptors (synthesized by cells bearing them) and cytophilic receptors passively acquired. This approach could also be useful in the study of immune system restauration using repopulated irradiated animals.

List of Publications

-"Importance of short lived lymphocytes in the immune response". V.Hooghe, G.Urbain-Vansanten, C.Richard and J.Urbain. Immunology (sous presse).

-"Cellular recognition and evolution". J.Urbain. Arch.Biol. (1974) 85,139.

-"On the high number of antigen binding cells in unimmunized mice".G.Urbain-Vansanten, C.Richard, V.Hooghe, C.Bruyns et J.Urbain.Ann.Immunol.(sous presse)

-"Isoelectric focusing of proteolytic fragments from rabbit anti-streptococcal antibodies of restricted heterogeneity". A.Vienne and M.Wikler. Febs Letters, vol.39, 160 (1974).

-"Temporal evolution of the sensibility to tetranitromethane of the active site of anti-tobacco mosaic virus antibodies". Immunology (sous presse). R.Jeener.

-"Linear and inverted repetitions in protein sequences". C.Wuilmart,L.Wyns and J.Urbain. J.Molec.Evolution (soumis à l'éditeur).

-"Antibodies to Micrococcus lysodeicticus : restricted structural heterogeneity in hyperimmunized rabbits". M.Van Hoegaerden, M.Wikler, R.Janssens, L.Kanarek. Eur.J.Biochem. (sous presse).

-"Isolation and characterization of homogenous rabbit antibodies to M. lysodeicticus with specificity to the peptidoglycan and to the glucose-N-acetyl aminomannuronic acid polymer". M.Wikler. Z.Immun. Forsch. (sous presse).

-"Hydrophobic properties and reactivity with lipids of the hinge region of immunoglobulins".Immunology (soumis à l'éditeur). R.Jeener.

The University of Dublin, Trinity College
Contract No. 130-74-1 BIOEIR
Professor F.G.A. Winder

STUDIES ON THE MECHANISM OF ACTION OF ENZYMES OF DNA REPAIR AND ON THEIR INDUCIBILITY

Mycobacterium smegmatis contains high activities of two enzymes believed to be involved in DNA repair. These are an ATP-dependent deoxyribonuclease, similar to the rec BC deoxyribonuclease of Escherichia coli, and a DNA polymerase, closely similar to DNA polymerase I of E.coli.

We have studied the properties and mechanism of action of the ATP-dependent deoxyribonuclease of this organism and compared its properties with those of the similar enzymes from several other bacteria as reported in the literature. It appears to differ from some of them in certain important respects, in particular in the role of ATP in interaction between the enzyme and DNA and in the nature of the reaction intermediates. However, it is not yet clear that these differences are not due to differences in techniques between laboratories.

This enzyme appears to have three distinct types of interaction with DNA, which we refer to as distinct complexes: a loose, ATP-independent interaction; ATP-dependent binding of DNA which is digested 'processively'; and ATP-dependent formation of a filter-bindable complex which is not digested processively. The physical nature of these complexes and their role in the action of the enzyme are still unclear.

Studies on the kinetics of complex formation and of DNA digestion, under a variety of conditions, suggest that both involve a common ATP-dependent process.

It has been shown that in M.smegmatis the two enzymes referred to in the first paragraph are synthesized in increased amounts following certain treatments which interfere with DNA synthesis. Such treatments include ultraviolet radiation, methyl methanesulphonate, nitrogen mustard, mitomycin C, hydroxyurea and iron limitation. On the other hand a number of agents

interfere with DNA synthesis without provoking this response, indicating that inhibition of DNA synthesis *per se* is not responsible. We have proposed that the effective treatments lead, directly or indirectly, to the production of damaged DNA and that this damage is detected by a monitoring system in the organism, which then responds by increased synthesis of these, and perhaps other, repair enzymes. This hypothesis is being tested.

This evidence for induction of repair enzymes in this organism is reminiscent of similar findings with the protozoan *Tetrahymena pyriformis*, obtained elsewhere. We are searching for similar phenomena in other prokaryotes and a eukaryote.

Project No. : 1
Research workers : Mr. T.F. Creedon, Dr. A.H. Johnson, Professor F.G.A. Winder
Title : Mechanism of action of a nuclease involved in radiation repair in bacteria

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The ATP-dependent deoxyribonuclease from Mycobacterium smegmatis had previously been partially purified and characterized. It had been shown to be closely similar in most properties to the rec BC deoxyribonuclease from Escherichia coli and similar enzymes from other bacteria. A model for the role of ATP in the enzyme's action had been put forward.

The enzyme appears to differ from some of the similar enzymes in certain important respects, and these have been investigated further. First, the enzyme forms only a loose and rapidly-reversible complex with DNA in the absence of ATP. This result has been obtained by three different techniques: membrane filter binding, DNA-agarose chromatography, and equilibration between enzyme-associated and free DNA. Secondly, we have been unable to find evidence for long single-stranded regions in a substantial proportion of DNA molecules undergoing digestion by the enzyme. Thirdly, contrary to some results with the E.coli enzyme, fairly large single-stranded pieces of DNA do not appear to be excised as intermediate products. However, it is still possible that some of the apparent differences between the enzymes are due to methodological differences between laboratories.

Three distinct types of interaction between the enzyme and DNA have been measured. One, which we refer to as complex A, involves that DNA which is broken down processively by the enzyme. The second, complex B, involves that DNA which is rendered membrane-bindable by the enzyme but whose breakdown by the enzyme can still be interfered with by other DNA. Formation of both complex A and complex B is ATP-dependent. In addition, a loose and rapidly reversible interaction between enzyme and DNA can occur in the absence of ATP.

However, the relationship between these complexes and the digestion of DNA by the enzyme is not clear. For example, although we can account for the kinetics of release of acid-soluble products by the enzyme in terms of a simple processive model in which, at 37°, the enzyme rapidly binds to free DNA chain ends and digests double-stranded chains of 15×10^6 daltons in about 90 sec, yet less than half of the DNA seems to be bound in complex A even when enzyme is in excess. Addition of an excess of unlabelled DNA to a reaction in progress leads to a more rapid decline in the rate of solubilization of labelled DNA than would be expected on the basis of this simple processive model.

Investigations at low concentrations of ATP indicate that complex A, once formed, is fairly stable in the absence of ATP. Increasing ATP concentration over the range 3 to 20 μM leads to sigmoidal responses in the rates of formation of complex A and of acid-soluble products, indicating that a cooperative interaction involving more than one molecule of ATP is involved in both processes. Analogues of ATP have parallel effects on formation of complex and on release of soluble products. These and other observations are most economically accounted for by assuming that digestion of the DNA is involved in the formation of the firm complexes.

Project No : 2
Research workers : Mr. A.W. MacNaughton, Mr. G.R. Campbell, Professor F.G.A. Winder
Title : Induction of enzymes related to DNA repair

Two enzymes which have been shown in other organisms to be involved in DNA repair are present in Mycobacterium smegmatis and are present in increased amounts following certain treatments which interfere with DNA synthesis. These enzymes are a DNA polymerase and an ATP-dependent deoxyribonuclease.

Treatments which increase levels of these enzymes include ultraviolet radiation,methyl methanesulphonate, nitrogen mustard, mitomycin C, hydroxyurea and iron limitation. Treatments which can selectively inhibit DNA synthesis without increasing the levels of these enzymes include nalidixic acid, 5-fluorouracil, ethidium bromide, acridine orange and caffeine. The results with the second class of treatments indicate that inhibition of DNA synthesis per se is not responsible for the increased enzyme levels.

Evidence has been obtained that the increased enzyme levels obtained by several of the treatments are due to increased enzyme synthesis rather than to activation of pre-existing enzyme. This is derived mainly from inhibition of these increases by chloramphenicol.

We have proposed that the first class of treatments leads directly or indirectly to the production of damaged DNA, and that this damage is detected by a monitoring system in the organism, which then responds by increased synthesis of these, and perhaps other, repair enzymes. Thus we postulate an inducible repair system in this organism.

In order to test this hypothesis,and, if it is correct, to determine the type or types of damage to DNA involved, we have had to devise a procedure for the gentle lysis of this organism under various conditions such as on sucrose gradients. This proved difficult because of their resistant walls and, further, even when cells lysed, DNA tended to remain with

residual cell structures, preventing its free sedimentation. However, suitable methods have now been developed and are currently being used.

We plan to extend this work to other prokaryotes and a eukaryote, Aspergillus nidulans. A.nidulans was chosen for several reasons, including the availability of mutants in DNA repair. This plan has necessitated investigations into the deoxyribonucleases of this organism, which had not been studied. Separation and partial characterization of four deoxyribonucleases and of a powerful inhibitor of one of them have been carried out. Marked changes in the activities of these enzymes with growth conditions and with state of growth of cultures have been demonstrated. No evidence for an ATP-dependent nuclease has been obtained, nor have we yet detected a nuclease specific for damaged DNA.

List of publications

1. A.W. MacNaughton and F.G. Winder, Increased deoxyribonucleic acid polymerase and adenosine triphosphate-dependent deoxyribonuclease activity in Mycobacterium smegmatis after treatment with alkylating agents, Biochem. Soc. Trans. 2 724-726 1974

2. T. Creedon, A.H. Johnson and F.G. Winder, Effect of ATP concentration on formation and breakdown of a complex between an ATP-dependent deoxyribonuclease and DNA, 9th FEBS Meeting, Budapest, Abstracts 147 1974

3. A.H. Johnson, T. Creedon and F.G. Winder, Complexes between DNA and the ATP-dependent deoxyribonuclease from Mycobacterium smegmatis, Biochem. Soc. Trans. in press.

Contractor: University College, Galway, Ireland.

Contract No.: 127-74-1 BIO EIR

Head of research team(s): Dr. James A. Houghton

General subject of Contract: The effects of radiation on the blue-green algae.

The blue-green algae are unique, they resemble higher algae in many aspects of their cell metabolism whilst being procaryotic, a characteristic shared only by bacteria. They occupy an intermediate position between the procaryotic bacteria and eucaryotic higher organisms and they share features common to both. They are consequently of immense phyllogenetic significance, nevertheless, very little successfulwork has been carried out on their genetics, progress being hindered by serious practical problems only now being overcome.

The blue-green algae are far more resistant than bacteria to UV irradiation and they also show extremely efficient photoreactivation. It has been suggested that they may have two photorecovery processes, one for the photoreactivation of pyrimidine dimers, the other for the repair of photosynthetic damage. The presence of a dark repair system has also been proposed. They show interesting responses to radiation and merit greater investigation than the limited studies conducted to date. In this study the effects of radiation, particularly UV, on two unicellular species, Gloeocapsa alpicola and Synechocystis pevalekii are being investigated. Their mechanisms for radiation protection and repair are being studied and also the mutagenic effects of radiation and its effects on genetic exchange.

Preliminary studies on the UV survival curves of Gleoecapsa have indicated that they are more resistant than bacteria and that photoreactivation of UV damage occurs.

Results of Project No. : 1.

Head of Project and scientific staff : Dr. James A. Houghton,
Professor L.K. Dunican, Dr. C.E. Buckley, Imelda Devilly.

Title of Project: The effects of radiation on Gloeocapsa alpicola.

Far UV Irradiation (λ300 - 350 nm)

The effects of far UV irradiation on G. alpicola were investigated and survival curves produced. Variation in the composition of the survival count or culture medium did not significantly alter the survival rate. However, holding the irradiated cells in liquid medium under white light for one hour prior to plating led to a marked increase in survival. In contrast, the survival of irradiated cells incubated in the dark for 24 hr. before transfer to normal growth conditions was reduced. Photoreactivation and dark repair in Gloeocapsa are now being investigated. The lighting conditions used for the growth of G. alpicola prior to UV irradiation was also found to markedly affect the survival pattern. Both the intensity and the wavelength of light used during growth were important factors. To establish whether the algal pigments were responsible the chlorophylls, carotenoids and phycocyanins of G. alpicola cells grown under different illumination conditions were studied. When the light intensity under which the cells were grown was decreased, the carotenoid content was markedly reduced and the UV survival rate was lowered. Using light of restricted wavelength also reduced the carotenoid level. Exposure of the cells to UV led to a 20% reduction in carotenoid content. By repeatedly subjecting cells to UV with a recovery period of 5 days between each irradiation UV resistant strains of G. alpicola were isolated showing a higher UV survival rate than normal. These strains showed a higher carotenoid content than the wild type. Even after prolonged exposure to UV the carotenoid contents were only slightly reduced. The possibility that carotenoids play an important role in conferring UV protection on blue-green algae is, therefore, feasible and is being investigated.

Near UV Irradiation (λ 254 nm)

It was found that near UV was much less effective than far UV in killing cells of Gloeocapsa alpicola. It was also observed that following irradiation with near UV, very efficient photoreactivation took place unless the cells were placed in the dark or under light not containing photoreactivating wavelengths. The maximum figure obtained for photoreactivation was 98% recovery. Warm white light (350-750 nm) and blue-light (300-550 nm) were found to be the most effective in photoreactivation, recovery rates of 93-98% after 24 hr. illumination being normal. Light of more restricted wavelength, e.g. pink, green, gold and red can be used to follow the decay in photoreactivation capability, after 16 hr. under light of non-photoreactivating wavelength photorecovery was lost. This very efficient photorepair of near UV radiation damage is in contrast to the much less efficient photoreactivation of far UV. This suggests that some irreversible damage is caused by far UV which does not occur with near UV. This would involve damage to the photosynthetic mechanism.

Mutagenesis

The mutagenic effects of UV radiation on G. alpicola are also being investigated. The techniques for mutant isolation have been developed using ethyl methane sulphonate and methyl methane sulphonate, both mutagens producing a high frequency of antibiotic resistant mutants in the surviving cells. Auxotrophic mutants have proved very difficult to isolate. It is also intended to study the effects of radiation on genetic exchange, particularly transformation, in G. alpicola.

Publications

Buckley, C.E. and Houghton, J.A. (1974). Some effects of UV irradiation on the blue-green alga, Gloeocapsa alpicola. Proc. Soc. Gen. Microbiol., 2, 20.

Contractors: 1. M.R.C. Cell Mutation Unit, University of Sussex, United Kingdom.

2. The Medical Biological Laboratory, T.N.O., Rijswijk, The Netherlands.

3. Department of Cell Biology and Genetics, Erasmus University, Rotterdam, The Netherlands.

4. Institute of Radiation Genetics and Environmental Mutagenesis, University of Leiden, The Netherlands

Contract No: 123-74-1 BIOC

Head of research teams: Professor D. Bootsma, Rotterdam, The Netherlands.

Professor B. A. Bridges, Sussex, United Kingdom.

General subject of contract: To identify and characterize variant strains of mammalian cells deficient in repair of DNA damage.

Normal mammalian cells can repair damage induced in their DNA by either UV-light, ionizing radiation or chemicals. In bacterial systems at least two dark repair systems have been characterized, excision repair and post-replication repair. Both may be error-prone, resulting in the appearance of gene mutations, but post-replication repair appears to be marked by the larger error-prone component. In mammalian cells the strongest evidence for the importance of an excision repair mechanism has come from the human hereditary skin disease xeroderma pigmentosum (XP) (Cleaver, J.E. 1970, J. Invest. Derm., 54, 181-195). Cells from XP patients seem to be defective in an initial step of DNA repair. By cell fusion techniques evidence was obtained suggesting different mechanisms were involved in different XP complementation groups (De Weerd-Kastelein, E.A., Keijzer, W., and Bootsma, D., 1972, Nature New Biol. 238, 80-83) but the steps of DNA repair affected by the different XP mutations have not yet been identified.

There is also evidence that normal mammalian cells have a repair mechanism which acts during or after normal DNA replication in S-phase (Lehmann, A.R., 1972, J. Mol. Biol. 66, 319-337; Buhl, S.N., Stillman, R. M., Setlow, R. B., and Regan, J. D. 1972, Biophys. J., 12, 1183-1191). At the commencement of this project, however, no mutant cell lines defective in post-replication repair had been identified.

The advances that have been made in the study of DNA repair processes in bacterial cells are in large measure due to the facility with which repair-deficient strains can be made and selected. It is the object of this contract to collect, isolate or construct mutants of mammalian cells with DNA repair deficiencies, particularly in excision repair and post-replication repair and in systems involved in repair of ionizing radiation damage, and to use the combined resources of the four participating laboratories to characterise these mutants as fully as possible both genetically and biochemically. Five different mutants have been observed in excision repair in human cells and the first human mutants deficient in post-replication repair were identified. In all cases these deficiencies resulted in the syndrome xeroderma pigmentosum. Evidence was obtained for differences in excision repair between human, chicken and Chinese hamster cells.

Results of Project No. 1:

Heads of Project and scientific staff: Dr. C. F. Arlett, Sussex
Professor D. Bootsma, Rotterdam
E. A. de Weerd-Kastelein
Rotterdam

Title of Project: Isolation of radiation-sensitive mutants

Two lines of Chinese hamster cells showing some UV sensitivity have been obtained from our standard V-79 stock. Biochemical and genetic characterization has not yet been completed.

Cultures of skin fibroblasts from three patients with a rare variant form of xeroderma pigmentosum (XP) have been obtained. These XP variant cells have been characterized as possessing a deficiency in post-replication repair.

Some classic excision-deficient XP lines have also been subjected to examination for the presence of other repair deficiencies.

Skin biopsies are being obtained from patients suffering from a number of other diseases with characteristics which might suggest deficiencies in DNA repair e.g., photosensitivity, X-ray sensitivity, proneness to recurring malignant disease, occurrence of chromosome aberrations. For example, cell strains from patients suffering from a progeroid syndrome (Hutchinson-Gilford, Hallermann-Streiff and Werner syndrome) were initiated. Cell strains from patients with the disseminated synthetic actinic porokeratosis (DSAP) syndrome were also obtained. It is indicated that sunlight plays a role in the etiology of this disease.

Results of Project No.2:

Heads of Project and scientific staff: Dr. A. R. Lehmann, Sussex
Dr. P. H. M. Lohman, Rijswijk
Dr. G. Veldhuisen, Rijswijk
Dr. R. R. Hewitt, Rijswijk
Dr. S. Bachetti, Rijswijk
Dr. M. M. Abboud, Sussex

Title of Project: Biochemical characterization of radiosensitive mutants

The repair of UV-induced DNA damage was studied in cultured human, Chinese hamster and chicken cells. UV-induced pyrimidine dimers were excised in human cells and to a lesser extent in Chinese hamster cells. In chicken cells pyrimidine dimers were found to be photoreactivated whereas excision of dimers in the dark did not occur. Photoreactivation was not observed in human and Chinese hamster cells. A non-dimer UV lesion was shown to be excised in chicken cells (see Paterson et al., Biophysical J. 14, 454-466, 1974).

In multinuclear heterokaryons obtained following fusion of chicken with human fibroblasts it was demonstrated that the chicken photoreactivating system could repair the UV-induced lesions in the human nuclei, and conversely the human excision repair system was able to cope with dimers in the chicken nuclei. (see Paterson et al. Biophysical J. 14, 835-845, 1974).

Experiments were started to isolate and characterize enzymes involved in both dimer and non-dimer repair processes in eukaryotic cells. In human and calf (thymus) cells a non-dimer dependent UV-endonuclease was found. Further characterization of the enzyme isolated from calf-thymus cells indicated that the purified enzyme exhibits activity against both UV- and gamma-irradiated DNA. However, the lesion recognized by the enzyme has not yet been identified.

Post-replication repair has been studied in four variants of the human disease xeroderma pigmentosum. These variant cell lines are all completely normal in their ability to carry out excision repair as judged by unscheduled DNA synthesis, repair replication and loss of UV-endonuclease-sensitive sites. They also are normal in their ability to join strand breaks produced by exposure to ionizing radiation.

All four, however, show an identical deficiency in post-replication repair. DNA synthesized after UV irradiation is of lower molecular weight than that in normal cells indicating the presence of daughter strand gaps and these gaps are joined very slowly. About eight hours are needed for gaps to be sealed to an extent which in normal cells is achieved in about two hours. This slow gap filling in XP variant cells is, moreover, almost completely inhibited by caffeine, a substance which has little or no effect on normal cells. A further, minor difference between normal and XP variant cells is that the latter synthesize DNA marginally more slowly after UV. Full details of these results will appear in the Proceedings of the National Academy of Sciences of the U.S.A.

In addition to the XP variant strains, some classical XP strains have also been examined and show an intermediate response between normal and XP variant strains, both for rate of daughter-strand gap filling and for caffeine sensitivity of this process. More classic XP cultures are being examined to see whether different behaviour may be ascribed to different complementation groups.

Results of Project No.3:

Heads of Project and scientific staff: Dr. C. F. Arlett, Sussex
Professor D. Bootsma, Rotterdam
Dr. J.W.I.M. Simons, Leiden
Dr. E. A. de Weerd-Kastelein

Title of Project: Genetic studies on DNA repair mutants

Survival studies:

Variant and classic XP cells, described in Project 1 and 2 have been examined for sensitivity to ultraviolet light (UV). The excision-deficient XP cells selected (belonging to complementation group C) were substantially more sensitive than normal cells whereas the variant cells were only slightly more sensitive. When caffeine was present after irradiation, however, both the XP variant and (to a slightly lesser degree) the classic XP cells were considerably more sensitive in contrast to normal cells which showed little or no increase in sensitivity. These observations correlate well with the biochemical results described in Project No.2.

Mutant studies:

Considerable effort is being put into the development of techniques for the quantitative induction of mutations in human cells and the greatest progress has been made in quantitating the induction of mutants deficient in hypoxanthine-guanine-phosphoribosyltransferase (HGPRT) which are resistant to the purine analogue 8-azaguanine.

The selection system requires several correction factors, which have been tested for their validity and now that experiments have been performed on the expression time of newly-induced mutants, the selection system is ready for operational use. The first small scale experiment for mutation induction by UV in wild-type human diploid skin fibroblasts indicates a linear dose response, with an induced frequency of $2{\cdot}2 \times 10^{-5}$ mutants per J m^{-2}.

Cloning efficiencies have been determined for several XP strains in order to select those which are most suitable for a mutation assay.

In addition the development of a method for studying repair during liquid holding of human diploid fibroblasts is in progress.

Much time is being devoted to the development of ouabain resistance as an alternative mutation assay system. In addition, with this system, we are attempting to circumvent the costly medium changes necessary when screening cells for 8-azaguanine resistance. Ouabain resistance works well with Chinese hamster and mouse lymphoma cells but has not yet been successfully applied to human fibroblasts under conditions where no medium changes are employed.

Cell fusion experiments

In collaboration with Dr. Jay Robbins, Bethesda, Maryland, five different complementation groups were observed in xeroderma pigmentosum, indicating the presence of five different mutations all affecting excision repair. These results were obtained by fusion of cells from different patients and the estimation of repair of UV damage in binuclear hybrid cells.

As shown in Project No.2 chicken cells most probably provide an excision repair mechanism which is different from excision repair in human cells. The data indicate that this chicken excision repair system does not recognize UV-induced thymidine dimers. Knowing that excision of thymine dimers is also absent in XP cells it was interesting to investigate complementation in chicken-XP binuclear cells. The Rotterdam part of the contract is restricted to the occurrence of unscheduled DNA synthesis in these cells, whereas the Rijswijk group investigates the excision of dimers by means of the UV-endo technique (see Project No.2). The first results indicate that fusion of chicken cells with XP cells of complementation group A followed by UV exposure did not result in normal levels of excision of dimers in the human nucleus. In the chicken nucleus only chicken type of excision repair was observed.

Contractor: The University College of Swansea

Contract No: 119-72-1 BIO UK

Dr James M Parry

Studies of the genetic, molecular and adaptive properties of RAD loci in yeast

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The well studied genetic system, wide range of radiation sensitive mutants and recently developed techniques of DNA labelling make the yeast Saccharomyces cerevisiae a convenient organism for both genetic and biochemical studies of the effects of radiation and chemical mutagen treatment.

Our research programme during 1974 has involved studies in yeast upon:-

a) The mechanisms of induced mutation, recombination and chromosome non-disjunction.

b) The influence of environmental factors such as the stage of cell division upon cell death and genetic change.

c) The nature of the repair defects in radiation sensitive mutants.

d) The analysis of single-strand break formation and DNA degradation after radiation treatment.

Some of the more important results include the demonstration of the role of post-replication repair in the production of mitotic recombinants and cell recover after radiation treatment.

Results of Project No: 1

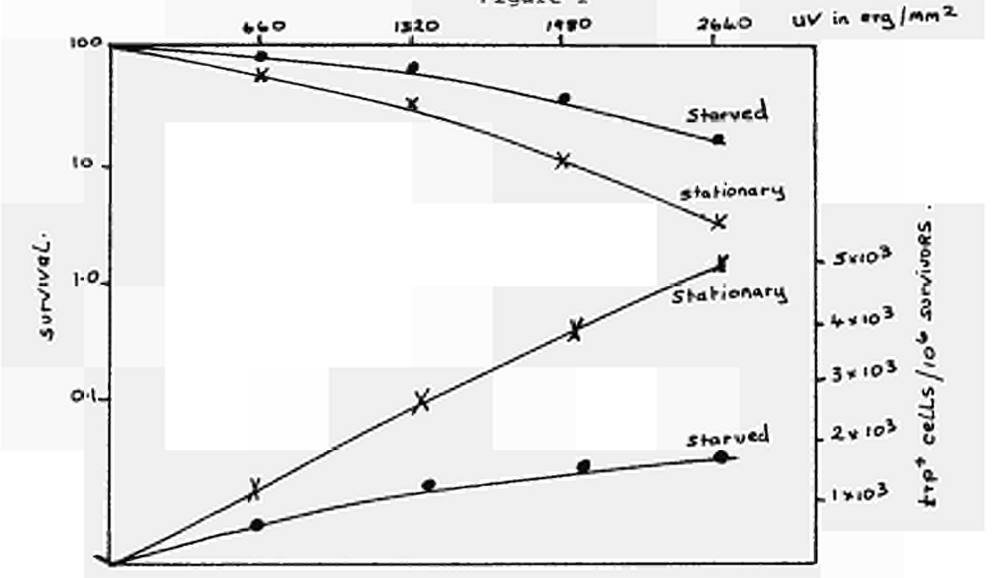
Head of Project and Scientific Staff: Dr James M Parry
Mr P J Davies

Title of Project: The effects of residual growth on induced mitotic gene conversion.

After mutagen treatment, stationary phase cultures of hetero-allelic diploid strains of yeast show residual growth for one or two divisions when plated upon selective minimal medium. In order to determine the effects of this residual growth upon the induction of mitotic gene conversion, stationary phase yeast cells were vigourously aerated for 16 hours in starvation medium in order to deplete the cellular pools of metabolites. In cultures given this treatment the residual growth of auxotrophic cells when plated on selective medium was abolished.

The effects of UV light exposure upon cell viability and mitotic gene conversion in stationary and starved yeast cells are shown in Figure I.

Figure I



The results demonstrate that starvation treatment prior to UV exposure results in a significant reduction in the yield of mitotic gene convertants accompanied by increases in the survival of irradiated cells at all UV doses.

Figure 2 and 3 demonstrate the effects of starvation treatment prior to nitrous acid and ethyl methane sulphonate (EMS) exposure.

Figure 2

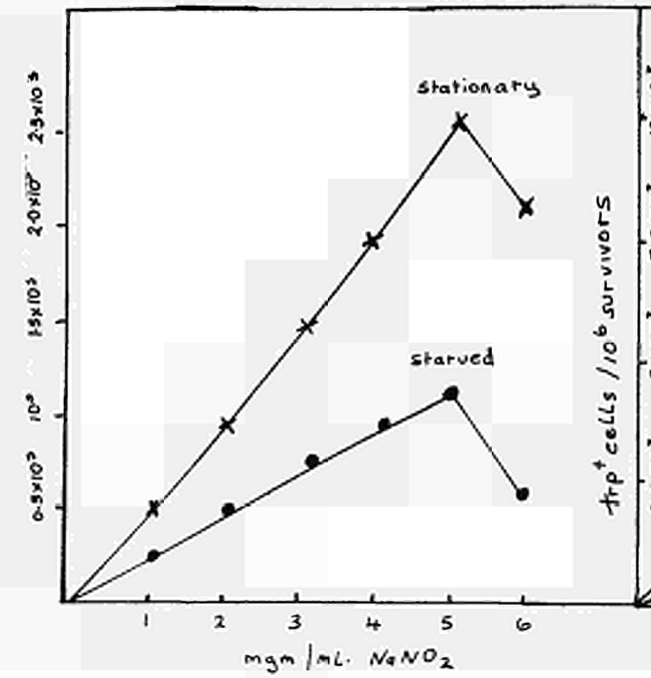
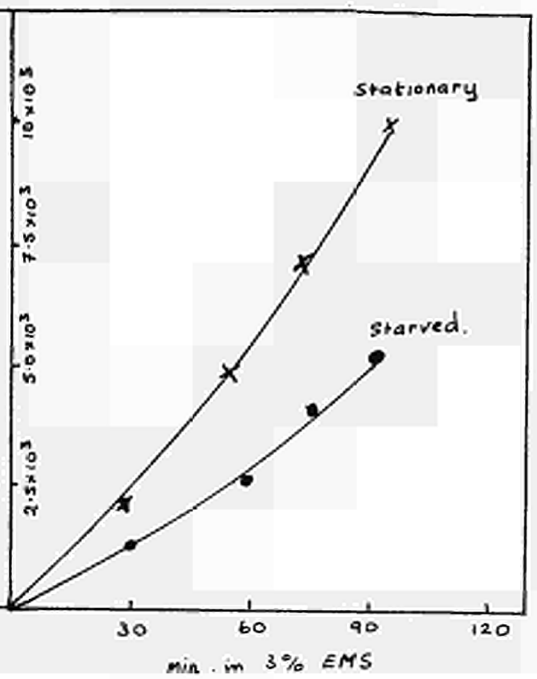


Figure 3



Both mutagens show reduced levels of mitotic gene conversion in starved cells compared to stationary phase cells. Starvation treatment also results in an increase in cell viability after nitrous acid treatment and a decrease after ethyl methane sulphonate treatment.

The reduced yield of induced mitotic gene convertants produced by starvation treatments suggests a correlation between residual cell division upon selective media and the induction of at least a fraction of the mitotic convertants produced by each of the three mutagen treatments. The results indicate that induced mitotic gene conversion results from the action of DNA repair enzymes acting upon single-strand breaks produced by DNA replication during the period of residual growth on selective medium.

Results of Project No: 2

Head of Project and Scientific Staff: Mr P J Davies
Dr James M Parry

Title of Project: The effects of caffeine treatment upon mutagen induced cell death, mitotic crossing-over and gene conversion in yeast

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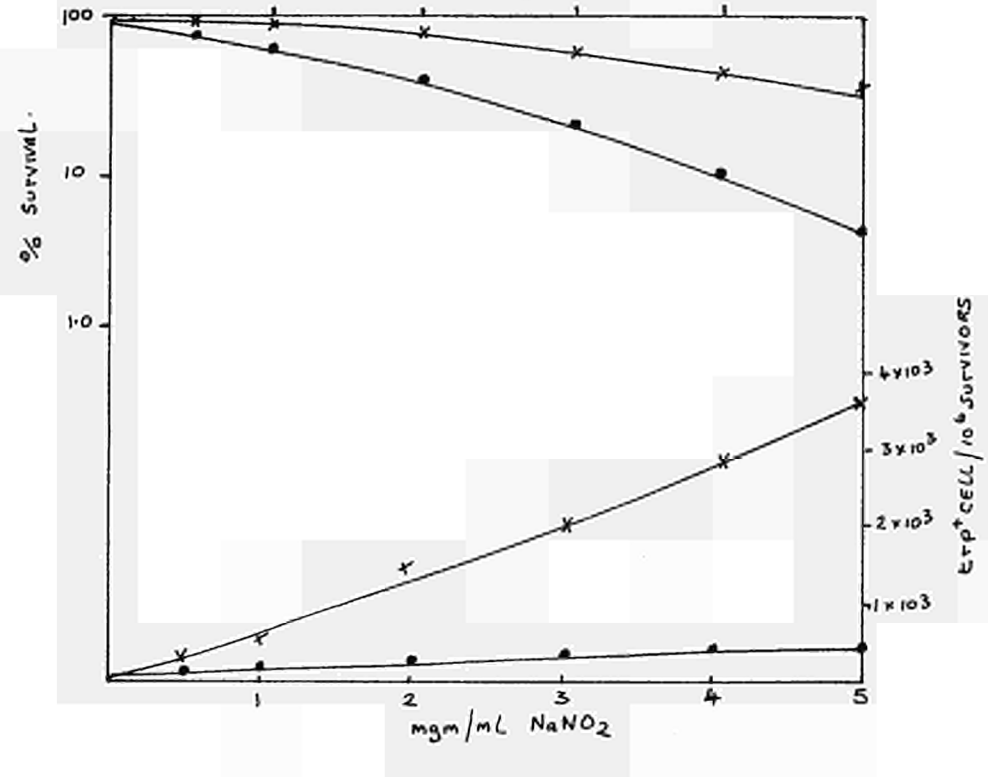
In eucaryotic cells, both direct biochemical evidence in mammalian cells (Lehman and Kirk-Bell 1974) and indirect studies in the yeast Schizosaccharomyces pombe using repair deficient mutants (Fabre 1972) suggests that caffeine inhibits a post-replication repair system which acts upon DNA lesions produced by mutagen exposure.

We have investigated the effects of caffeine treatment upon cell death, mitotic gene conversion and mitotic crossing-over induced by UV light, nitrous acid and ethyl methane sulphonate in diploid cultures of Saccharomyces cerevisiae. In all the experiments the caffeine treatments were performed by incorporating 0.3% caffeine into the complete and selective plating medium.

After all mutagen treatments producing cell death, caffeine treatment results in a reduction in cell viability compared to plating upon nutrient media alone. As shown in Figure 1, caffeine treatment after exposure to 5 mgm/ml of sodium nitrite results in a reduction in cell viability from approximately 30% to 8%.

Figure 1



All three mutagen treatments also produce an increase in the frequency of homozygous recessive colonies produced by mitotic crossing-over. When mutagen treated cells are plated upon media containing caffeine this increase in mitotic crossing-over was completely abolished after all mutagen treatments.

The most dramatic response to caffeine observed in our experiments was its effect upon mitotic gene conversion induced by nitrous acid and ethyl methane sulphonate. As shown in Figure I after treatment with 3 mgms/ml of sodium nitrite plating upon selective medium containing caffeine results in a reduction in gene conversion at the heteroallelic tryptophan-5 locus from 360 prototrophs/10^5 cells to 30 prototrophs/10^5 cells. In contrast to the reduction in mitotic gene conversion produced by caffeine after nitrous acid and ethyl methane sulphonate treatment, no such effect could be detected after UV exposure.

The results obtained implicate the action of a caffeine sensitive post-replication repair process in the production of mitotic crossing-over after treatment with all three mutagens and the production of mitotic gene convertants after nitrous acid and ethyl methane sulphonate treatment.

Results of Project No: 3

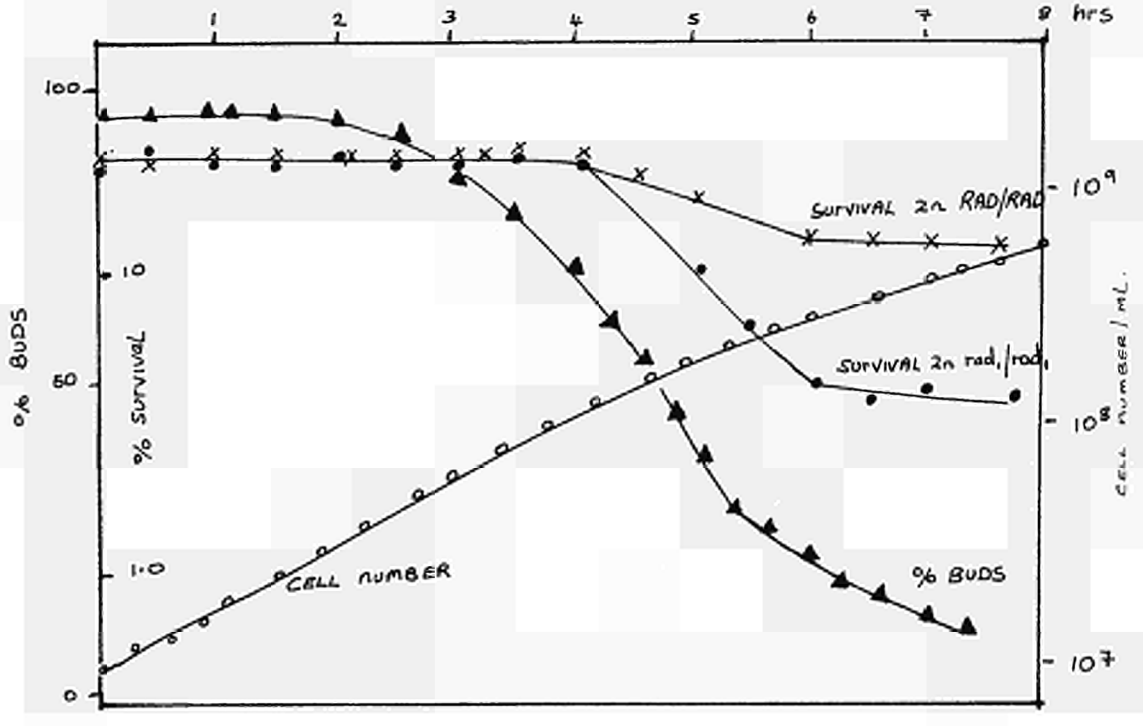
Head of Project and Scientific Staff: Dr James M Parry
Mr P J Davies
Dr W B Evans

Title of Project: The variation in UV sensitivity of yeast cultures during cell division.

Growing cultures of Saccharomyces cerevisiae show increased sensitivity to UV light and ionising radiation during the stationary phase of the growth cycle. The changes have been correlated with an increase in the frequency of budding cells during the resistant exponential phase of growth. In order to investigate these changes in more detail we have examined the effects of UV light upon yeast cultures as they progress from the exponential to the stationary phase of growth.

Figure I demonstrates the transition over an 8 hour growth period of a yeast culture from exponential to the stationary phase of growth, during which period cell number increases from 10^7 to 3×10^8 cells/ml.

Figure I



The sensitivities of a wild type diploid culture of yeast to two doses of 1320 and 2640 ergs/mm^2 respectively of UV light are shown in Figure I. The results demonstrate that the wild type culture is resistant to UV exposure during the exponential phase of growth with an increase in sensitivity at approximately 4 hours. This period of increased UV sensitivity corresponds with the end of the transition period. The frequency of budding cells in the population shows a significant reduction at least one hour before the increase in sensitivity to UV light.

Figure I also demonstrates the variation in UV sensitivity of an excision deficient diploid yeast culture at a UV dose (280 ergs/mm^2) which produces an equivalent reduction in survival to 2640 ergs/mm^2 in a wild type culture during exponential growth. The results demonstrate that the excision deficient strain of yeast is significantly more sensitive to UV light during the stationary phase of growth than the wild-type culture at comparable UV doses.

The results thus implicate a repair system other than excision-repair in the cell cycle induced changes in UV resistance. These changes in resistance are being investigated further by studies of the rates of cellular repair and macromolecular synthesis in cells at different stages of cell growth.

Results of Project No: 4

Head of Project and Scientific Staff: Dr Elizabeth M Parry
Dr James M Parry

Title of Project: Macromolecular synthesis after UV exposure in excision defective cultures of yeast.

Compared to wild type cultures, excision-deficient mutants of yeast show much increased resistance to UV light during the exponential as compared to the stationary phase of growth. We have investigated further the resistance of excision-deficient strains of yeast during exponential growth by detailed studies upon macromolecular synthesis after UV exposure.

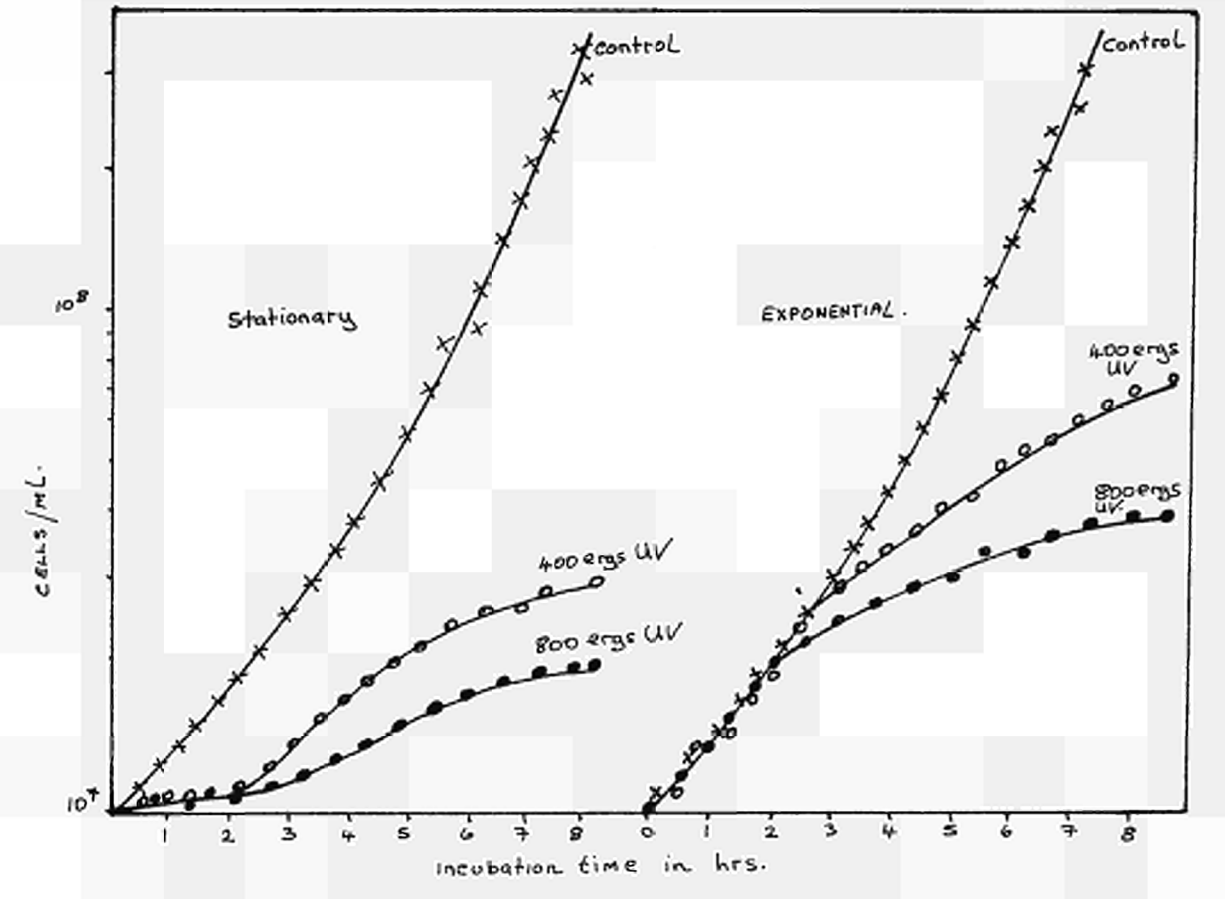
Unirradiated cultures of the excision-deficient diploid mutant e_9/e_9 show little or no growth delay when either stationary or exponential phase cells are transferred to fresh growth medium. Such cells show increases in DNA, RNA and protein content until they reach a period of maximum growth rate after 5 hours incubation, when they achieve a doubling time of approximately 75 mins.

UV exposure of stationary phase cultures of e_9/e_9 results in a growth delay of $1\frac{1}{2}$ to 2 hours after 400 and 800 ergs/mm^2 respectively. The irradiated cultures grow slowly until cell number reaches a plateau after approximately 6 hours of incubation. At this time 800 ergs/mm^2 of UV exposure reduced the final yield of cells by approximately 10 fold.

In contrast, as the results in Figure 1 demonstrate no growth delay could be detected when exponential phase cells of e_9/e_9 were exposed to 400 and 800 ergs/mm^2 of UV light. In our experiments no differences in the rates of macromolecular synthesis could be detected between irradiated and unirradiated cells over the first $2\frac{1}{2}$ hours of incubation. After this period reductions in the rates of growth of irradiated cells were detectable until both irradiated cultures show a plateau in the yields of cells at approximately 6 hours.

The results obtained indicate that in yeast cultures defective in excision repair a significant contribution to the viability of UV irradiated cells is made by a post-replication repair process which takes place in actively dividing cells. Preliminary experiments in which UV induced recombination has been measured in stationary and exponential cells of e_g/e_g have demonstrated increased rates of mitotic crossing-over in the UV resistant exponential phase cells.

Figure I



Results of Project No: 5

Head of Project and Scientific Staff: Dr James M Parry
Mr P J Davies
Dr W B Evans

Title of Project: The sensitivity of yeast cultures to chemical mutagens during cell division

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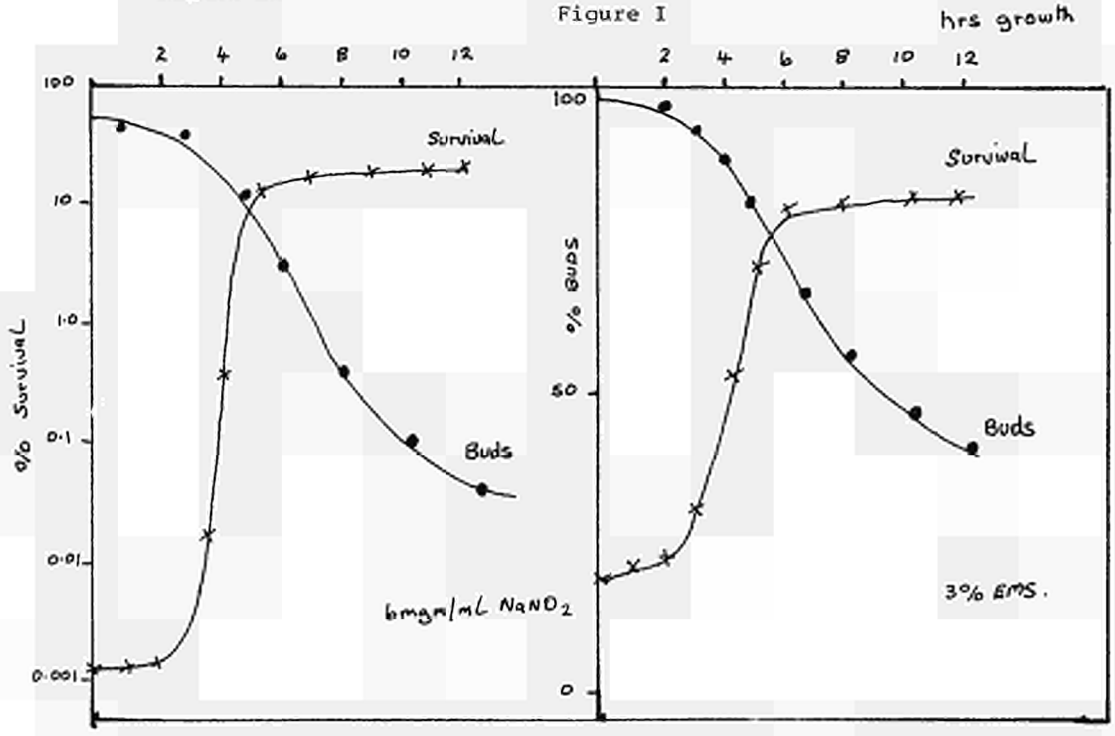
Unlike their response to UV light and ionising radiation yeast cultures show increased sensitivity to chemical mutagens during the exponential phase of growth. Treatment of haploid and diploid yeast cultures with nitrous acid and ethyl methane sulphonate (EMS) during the transition from exponential to the stationary phase of growth reveals a significant rise in resistance to cell killing at an early stage in the transition period, as shown in Figure I.

Figure I



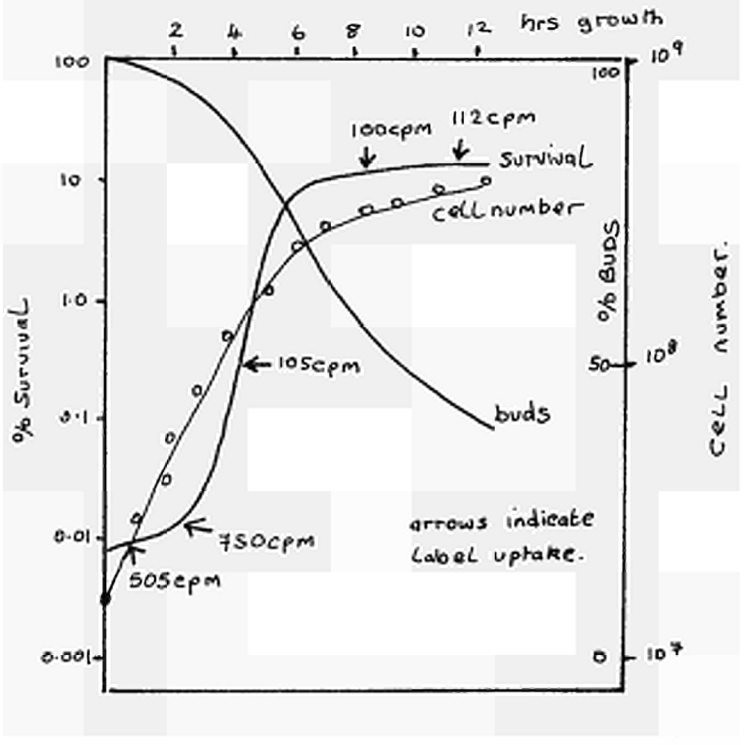
The results shown in Figure I demonstrate that after exposure to 6 mgs/ml of sodium nitrite and 3% EMS cell resistance increases by approximately 4 log cycles and 3 log cycles respectively during the transition period. This increase in resistance takes place at approximately 3 hours during which time cell division is actively taking place and correlates with the start of the period

during which the frequency of budding cells falls. We have previously shown that this period correlates with changes in the yeast cell wall as detected by reduced sensitivity to snail gut enzyme.

The results suggest that at least a fraction of the growth induced changes in sensitivity to chemical mutagens described here depend upon variation in the uptake of the individual mutagens. This hypothesis was investigated further by the use of radioactively labelled EMS.

Yeast cells growing in the transition period from exponential to the stationary phase of growth were exposed to radioactive EMS in the presence of an excess of unlabelled mutagens. The results of a typical experiment are shown in Figure 2 and demonstrate that approximately 4 times the amount of ^{14}C labelled EMS is taken up by yeast cells during the exponential compared to the stationary phase of growth. The reduction in uptake of ^{14}C labelled EMS corresponds closely with the increase in resistance to cell killing detected during the transition. These experiments are being extended to include the effects of culture age upon the interactions between radiations and chemical mutagens.

Figure 2



Results of Project No: 6

Head of Project and Scientific Staff: Dr James M Parry
Mr P J Davies

Title of Project: The induction of mitotic gene conversion yeast cultures from the stationary and exponential phase of growth

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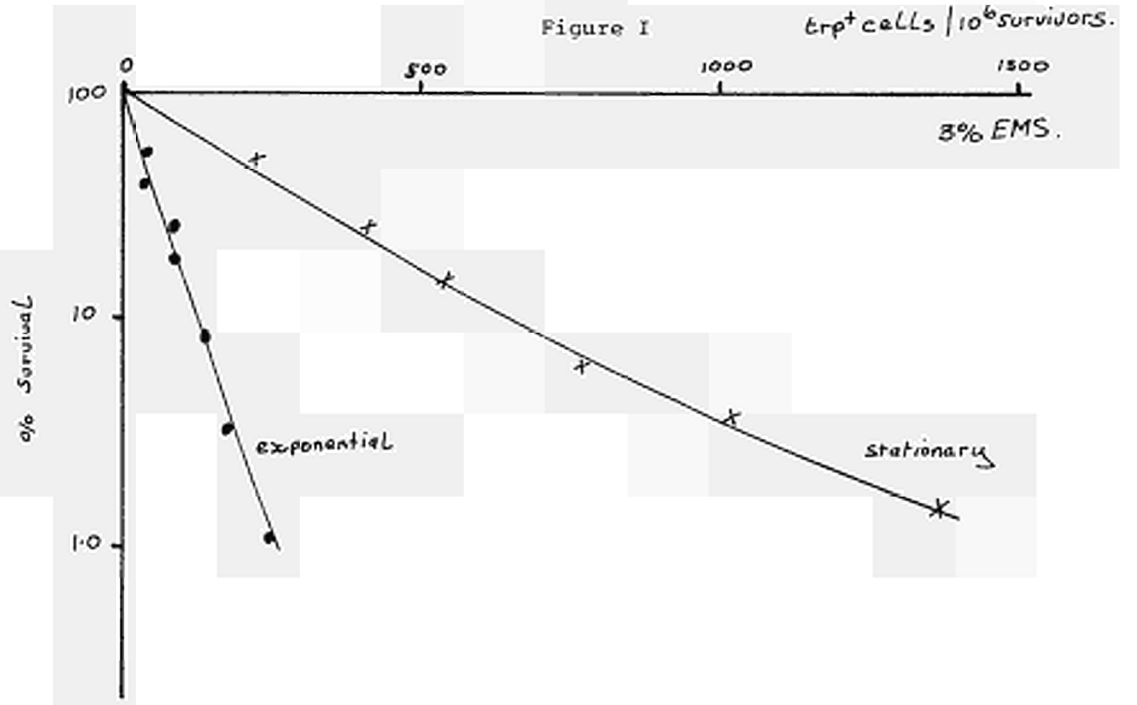
In a previous report we described the variation in the sensitivity of yeast cells to radiation treatment and chemical mutagens. We have extended these observations to include measurements of the frequency of mitotic gene conversion induced in heteroallelic diploid cultures of yeast after treatment of exponential and stationary phase cultures with UV light, nitrous acid and ethyl methane sulphonate.

When yeast cultures are treated with nitrous acid or ethyl methane sulphonate maximum induction of mitotic gene conversion is found in stationary phase cultures which show the maximum resistance to cell death. Figure I demonstrates the effect of 3% ethyl methane sulphonate upon mitotic gene conversion as a function of cell viability. The results clearly demonstrate the increased induction of gene conversion in stationary phase cells amongst the cells surviving mutagen treatment. The results indicate that during the resistant phase of growth more recombination takes place leading to gene conversion and cell viability.

Our data, combined with the previous experiments involving measurements of the uptake of chemical mutagens suggests that the resistance of stationary phase cells results from both reduced mutagen uptake and increased post-replication repair activity.

In contrast to the results obtained with chemical mutagens, yeast cells show increased resistance to UV light during the exponential phase of growth. When frequency of gene conversion was measured after UV exposure of cells from the exponential and stationary phase of growth a significantly higher frequency of convertants were detected in the stationary phase of growth. Thus unlike chemical mutagens the maximum frequency of convertants after UV exposure is produced during the most sensitive stage of growth.

Figure I



When the frequency of UV induced gene conversion was plotted on the basis of the yield of convertants against cell survival the results were found to fall on the same curve. Thus irrespective of the sensitivity of a yeast culture to UV exposure, the yield of recombinants produced by gene conversion is identical.

Results of Project No: 7

Head of Project and Scientific Staff: Dr James M Parry

Title of Project: The induction of mitotic non-disjunction in yeast

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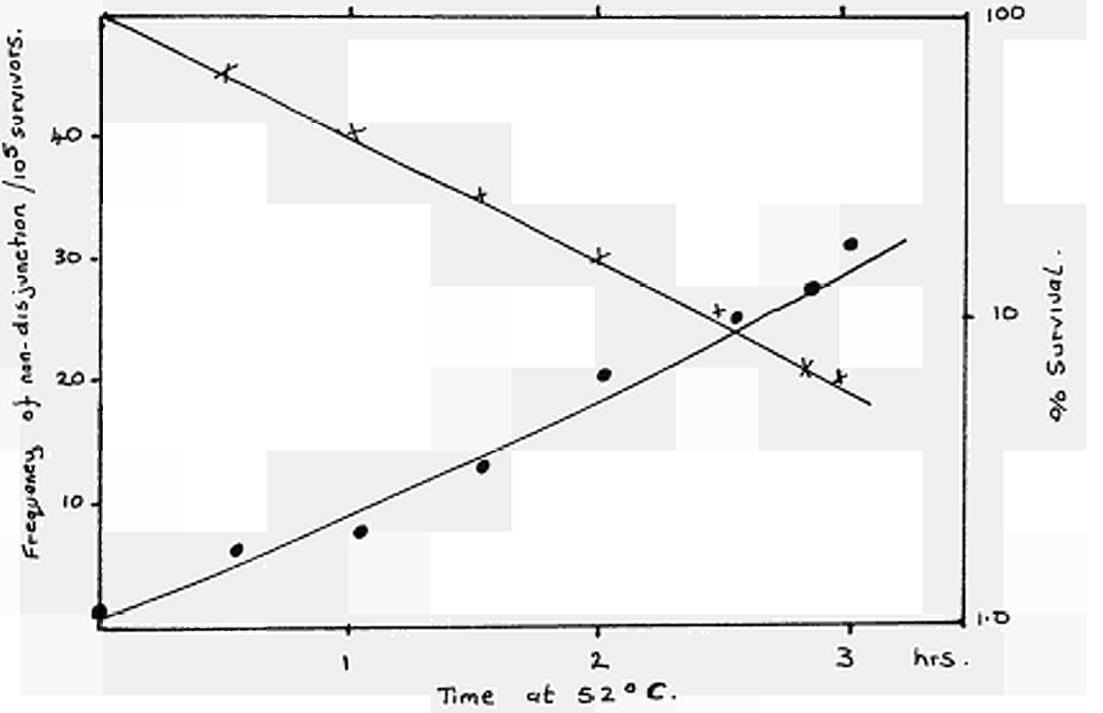
In a previous report we described the induction of mitotic non-disjunction in cultures of yeast after exposure to ionising radiations. A considerable improvement in the detection of mitotic non-disjunction has become possible because of the availability of a yeast strain D_6, developed and supplied by Dr F Zimmermann. By the use of selective medium containing the antibiotic cyclohexamide, white adenine requiring cyclohexamide resistant cell produced by mitotic non-disjunction of Chromosome IV may be detected.

D_6 has been used to determine the frequency of 2n - 1 cells produced after treatment with a number of agents including gamma irradiation, heat shock at 52°C and pH change. Immediate plating of treated cells upon selective medium after exposure to the various agents failed to produce 2n - 1 cells. After inducing treatment, cells of D_6 were innoculated into nutrient medium and incubated to allow at least two cell divisions to take place before plating upon selective agar. Under these conditions 2n - 1 cells produced by mitotic non-disjunction were detectable after all three inducing treatments.

The results of treatment at 52°C upon the frequency of 2n - 1 cells after growth in nutrient medium to allow for expression are shown in Figure I. The results demonstrate that the frequency of white cyclohexamide resistant cells increase with 52°C treatment from a spontaneous frequency of $1/10^5$ cells to $30/10^5$ after 3 hours heat treatment.

Similar increases in the frequency of mitotic non-disjunction were produced after allowing for expression in nutrient medium after inducing treatment with gamma irradiation and after growth at acid pH.

Figure I



These studies are being extended to determine the period of maximum sensitivity during cell growth to the induction of mitotic non-disjunction by ionising radiations. Similar selective systems are being developed which allow the detection of radiation induced meiotic non-disjunction.

Results of Project No: 8

Head of Project and Scientific Staff: Dr Elizabeth M Parry
Mr S Piperakis

Title of Project: Temperature sensitive radiation sensitive mutants of yeast

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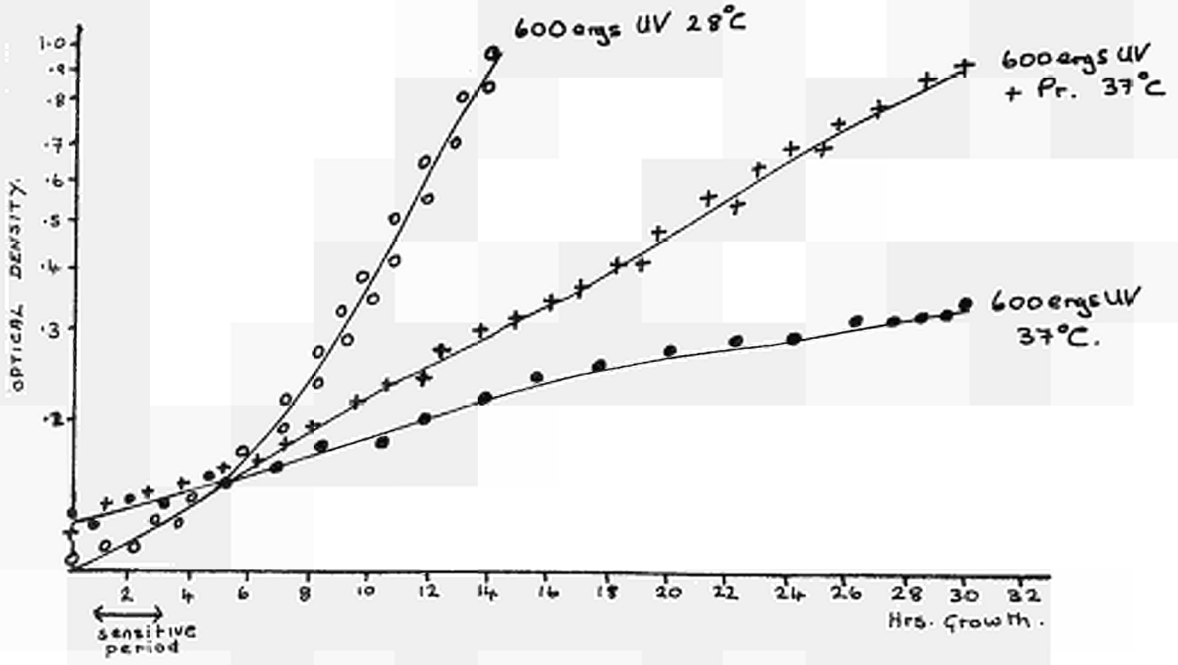
Approximately 200 yeast mutants characterised by their sensitivity to UV light exposure at 37°C and wild type resistance at 28°C are being studied. A number of such mutants show cross-sensitivity to nitrous acid treatment while others are cross-sensitive to ionising radiations and alkylating agents. On the basis of the sensitivity of the excision defective mutants $\underline{rad}_1$ $\underline{rad}_2$ and $\underline{rad}_3$ to nitrous acid treatment the members of the former group are classified as presumptive conditional lethal excision-repair mutants.

Genetic analyses are in progress and so far all the mutants studied behave as independent Mendelian genes. Complementation tests are being used in order to classify the individual mutants into functional groups. Complementation tests using the ts rad mutants mated to the radiation-sensitive rad mutants have shown a high frequency of complementation for UV resistance between the two mutant groups. Thus only a limited number of ts rad mutants have so far been classified as conditional lethal mutants of the classic rad loci of yeast.

Studies of macromolecular synthesis have been performed, before and after mutagen treatment at both permissive and restrictive temperatures. After UV light treatment the mutant ts rad 102 shows wild type increases in cell number, DNA, RNA and protein synthesis at 28°, whereas macromolecular synthesis ceases at 37° immediately after UV exposure. No evidence of DNA degradation at 37° was detectable in this mutant thus implicating the involvement of post-UV cell division in the process of DNA degradation.

It is envisaged that the potential of the ts rad mutants lies in the determination of the time of action of the enzymes involved by the use of temperature switch experiments. The growth of one mutant ts rad 722 has been studied in this way before and after UV and gamma irradiation. Whereas wild type yeast cells show a lag in growth at 37°C both with and without radiation treatment, cells of ts rad 722 show a similar lag at 37°C without irradiation but after UV exposure or gamma irradiation cell growth is inhibited in a dose dependent manner. The behavior of ts rad 722 after 600 ergs/mm^2 of UV light is shown in Figure I.

Figure I



As shown in Figure I cell growth of ts rad 722 at 37°C does take place if the culture is exposed to visible light treatment after UV exposure. Thus indicating that there is a photo-reactivable sector to growth inhibition at 37°C i.e. UV induced pyrimidine dimers are implicated in the process. Temperature switch experiments with ts rad 722 showed that after 24 hours the destiny of the cells was determined and could not be influenced by a change of temperature. These temperature switch experiments were performed at various times until it was found that a critical growth period exists during the period 1 to 3 hours. If cells were to survive radiation treatment they must be grown at 28°C during this period and subsequent switches do not alter their destiny.

The results obtained with ts rad 722 indicate that during the period 1 to 3 hours a specific repair enzyme is synthesised or acts upon radiation induced DNA lesions. The two alternatives may be distinguished by further investigations of the behavior of ts rad 722 in the presence of inhibitors of protein synthesis.

Results of Project No: 9

Head of Project and Scientific Staff: Dr W E Evans
Dr P Wilmore
Dr James M Parry

Title of Project: DNA degradation after mutagen treatment in yeast

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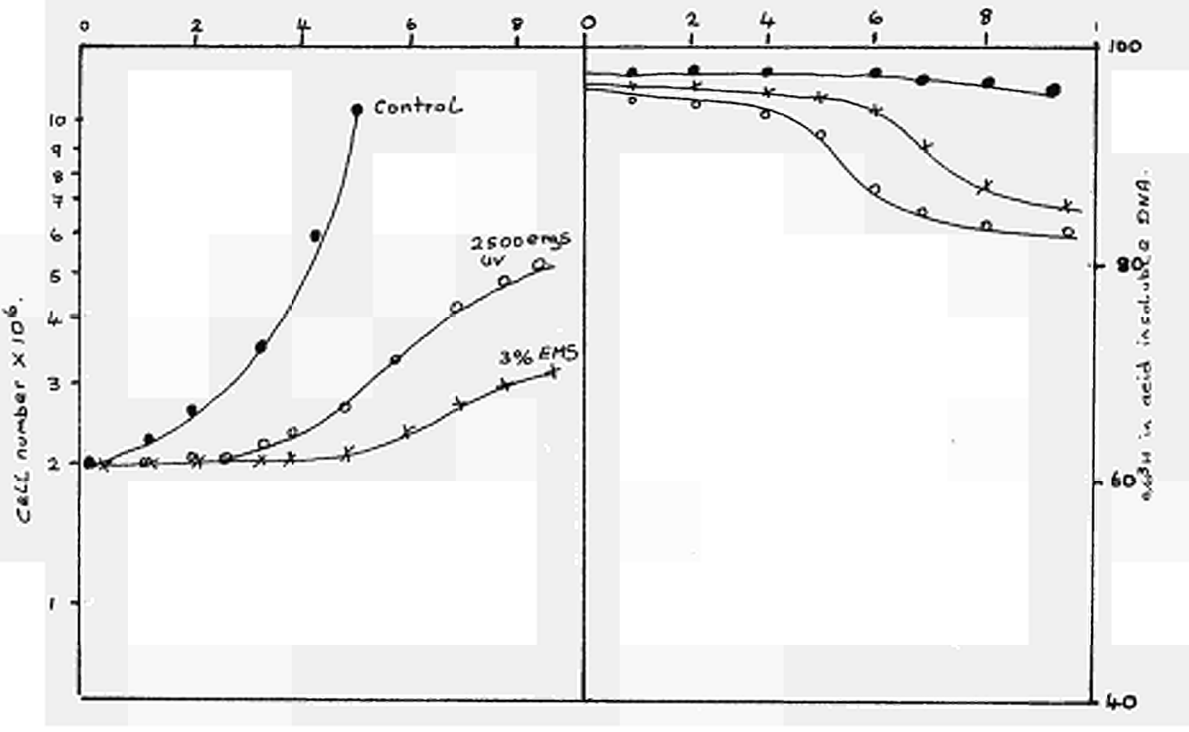
The availability of mutant cultures of yeast capable of incorporating the specific DNA label thymidine monophosphate have enabled us to initiate an investigation into the behavior of DNA after mutagen treatment.

The yeast strain tup-2 (Wickner 1974) incorporates exogenous ^{3}H-thymidine monophosphate in the presence of aminopterin and sulphonamide. Cultures labelled in this manner were exposed to a range of inactivating treatments, including UV light, gamma irradiation, ethyl methane sulphonate (EMS) and heat shock at 52°C. Treated cells were incubated in growth medium for periods of up to 12 hours. During this period the % of radioactivity remaining in the acid insoluble DNA and the rates of cell division were determined. The results of some typical experiments are shown in Figure I.

Figure I



In the control culture, cell division commences approximately 1 hour after incubation and the culture enters the exponential period of growth after 3 hours. In contrast, as the results in Figure 1 demonstrate the mutagen treatments produce significant delays in the initiation and rates of cell division. For example treatment with 30 Krads of gamma rays results in a division delay of 5 hours whereas 120 mins exposure to 3% EMS delays the start of growth for 8 hours after incubation.

Figure I also demonstrates that the mutagen treatments also result in the loss of radioactive material from the acid insoluble DNA of treated cells. This degradation of DNA which may involve the loss of up to 20% of the prelabelled material occurs only in dividing cells after a period of mutagen induced growth delay.

Similar experiments have been performed using the tmp1-1 mutant supplied by Dr M Brendel which has a specific requirement for thymidine monophosphate. In this mutant UV induced DNA degradation may also be detected but cultures of this mutant are characterised by extended periods of division delay after UV exposure.

These experiments are at present being continued using petite and grande cultures of yeast carrying repair deficient mutations.

Results of Project No: 10

Head of Project and scientific staff: Dr James M Parry
Dr W E Evans

Title of Project: The induction of DNA single-strand breaks by mutagen treatment in yeast.

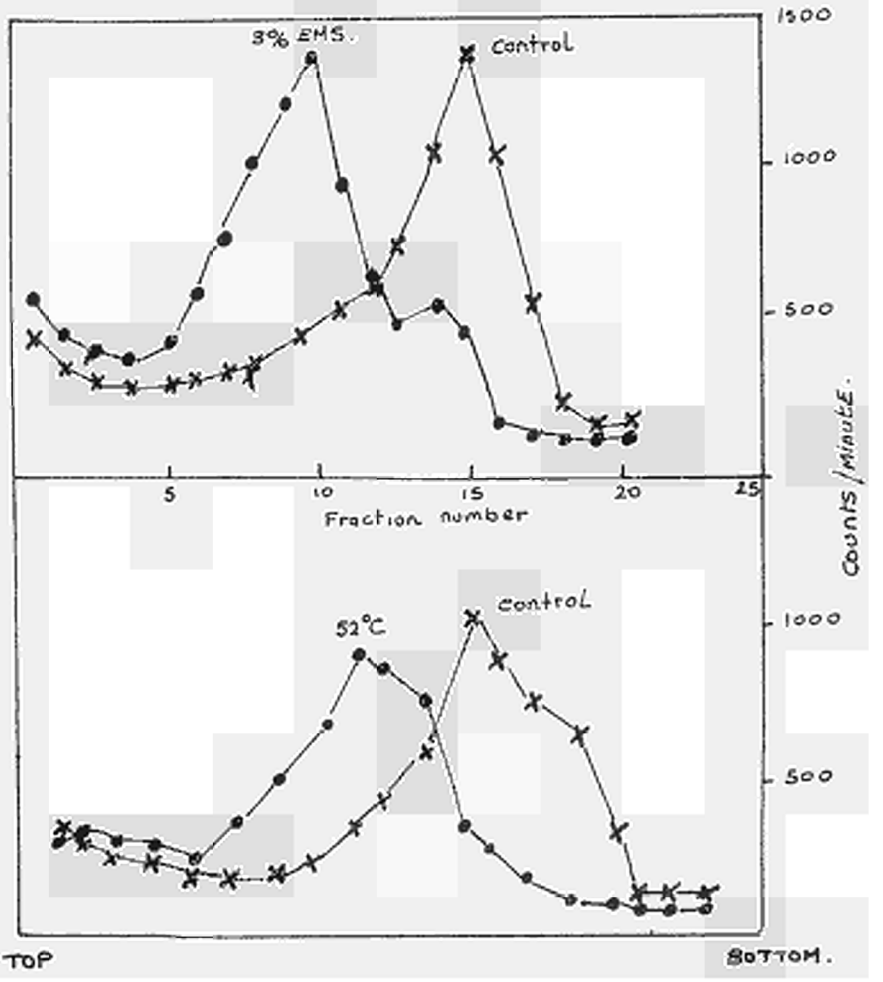
DNA strand breaks are amongst the most common lesions induced by ionising radiation. Their role in the inactivation of bacteria has been extensively reviewed by Town (1973). The analysis of radiation induced strand breaks and their repair has been studied in a wide range of organisms by the use of alkaline sucrose gradient analysis but at the present time only a limited amount of data is available concerning the yeast Saccharomyces cerevisiae.

We have initiated an investigation into the possible value of alkaline sucrose gradient analysis in a study of the role of DNA strand breaks in the inactivation of yeast cells exposed to a range of mutagenic treatments. These experiments have involved the use of ^{3}H Uracil as a general nucleotide label and yeast cells were treated with mutagen after treatment of log phase cells with snail gut enzyme to produce sphaeroplasts. After sedimentation in 5 - 20% alkaline sucrose gradients fractions were collected and hydrolysed in 0.3N sodium hydroxide to remove RNA. Acid insoluble precipitates were counted to determine the position of DNA in the gradients with and without mutagen treatment.

Figure 1 demonstrates the results typical experiments of the treatment of yeast cells with 3% ethyl methane sulphonate and heat shock at 52^{o}C upon the position of radioactive DNA after sedimentation on alkaline sucrose gradients. The results demonstrate that both ethyl methane sulphonate and heat shock produce decreases in the molecular weight of treated DNA indicating the induction of single strand breaks after mutagen exposure

These experiments are being extended to the use of ionising radiation treatment in both wild type and repair deficient mutants of yeast. Such experiments will enable us to determine the induction and repair of single strand-breaks after radiation exposure.

Fig. 1.


3% EMS.
Control
1500
1000
500
5
10
15
20
25
Fraction number
Counts / minute.
52°C
Control
1000
500
TOP
BOTTOM.

Results of Project No: 11

Head of Project and Scientific Staff: Dr James M Parry

Title of Project: Cell death and DNA repair systems of yeast

The yeast mutant rad-15 shows increased sensitivity to UV and γ irradiation and is characterised by a loss of viability when stored in saline after UV exposure. During this period of storage the photoreactivable sector of UV damage is reduced indicating the loss of UV induced pyrimidine dimers. During quantitative estimations of this process of negative liquid holding recovery a sub-culture of rad 15, coded rad 15-d was isolated, which unlike wild type cultures showed a loss of cell viability when stored in saline even in the absence of UV exposure.

The nature of the loss of viability, shown by rad 15-d was studied further by an investigation into the effects of variation in both culture age and the temperature of both growth and saline incubation. When stored in saline at 28°C, cultures of rad 15-d grown for up to 8 days on nutrient agar show progressive cell death down to 0.001% viability after 20 days incubation. In contrast the loss of viability in a related wild type culture was approximately 50%. Although rad 15-d shows normal wild type growth at 37°C cultures held at this temperature in saline show greatly accelerated cell death with a complete loss of viability after 5 days incubation at 37°C. The sensitivity of rad 15-d to "saline death" is also increased in older cultures harvested from nutrient plates. After 18 days on nutrient agar the culture shows a loss of viability down to 0.001% survival after 8 days incubation at 28°C.

Cells of rad 15-d were mated to a wild type haploid strain of yeast to produce a heterozygous diploid culture that was resistant to gamma irradiation and showed only wild-type loss of viability during long periods of saline incubation. After sporulation, tetrads were separated and analysed for gamma sensitivity and "saline death". In all the spores examined, gamma sensitivity segregated in 2 of the 4 spore products with the phenotype "saline death". The data thus indicates that the two phenotypes gamma sensitivity and "saline death" result from the action of single recessive Mendelian genes.

The properties of rad 15-d are similar to those of yeast mutants isolated on the basis of their sensitivities to heat shock at 52°C (Evans and Parry 1974). Mutants of this type also show accelerated "saline death" and are cross-sensitive to ionising radiations and UV light. The results obtained with both types of mutants suggest a correlation between cell death under non-nutrient conditions and the presence or absence of enzyme systems capable of repairing radiation induced cell damage.

Results of Project No: 12

Head of Project and Scientific Staff: Dr James M Parry
Dr Elizabeth M Parry

Title of Project: The genetic effects of UV light and liquid holding treatment in excision defective strains of yeast

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Wild type cultures of yeast show an increase in cell viability after UV exposure if cultures are held in saline for a number of days before plating upon nutrient medium. In contrast haploid mutants of yeast defective in the genes regulating excision repair, rad-1, rad-2 and rad-3 show negative liquid holding, a reduction in viability after UV exposure, implicating the excision repair system in liquid holding recovery and that cell lethality results from a decline in the activity of a post-replication repair system.

Previous experiments have shown that in homozygous diploid cultures carrying mutant alleles of rad-1, rad-2 and rad-3 show no change in cell viability after liquid holding treatment. The lack of viability changes in such excision defective diploids may be explained by at least two possibilities:-

a) That no change in repair activity occurs before or after liquid holding treatment.

b) A reduction in the activity of a post-replication repair system does occur, but its activity is replaced by the action of an additional mechanism found only in diploid cultures of yeast.

If no change in repair activity occurs during liquid holding treatment in excision defective diploid cultures we would predict that the levels of UV induced mitotic recombinants and mutation to

prototrophy will remain constant before and after such treatment. In order to test these two alternatives, a number of homozygous diploid cultures of rad-2 and rad-3 were constructed with genotypes suitable for the detection of recombinants produced by mitotic crossing-over and mutation to prototrophy.

When diploid cultures homozygous for either rad-2 or rad-3 and carrying the homozygous marker adenine-2 were exposed to UV irradiation the frequency of adenine independent prototrophs increases to 220 prototrophs/10^5 viable cells at 250 ergs/mm^2 in the rad-2 culture. Liquid holding treatment after UV exposure results in a significant reduction in prototroph frequency to 12 prototrophs/10^5 viables at 250 ergs/mm^2 with no change in cell viability. Similar results were obtained when the experiments were performed in rad-2 and rad-3 cultures which produce homozygous adenine requiring recombinants by a process of UV induced mitotic crossing-over. In rad-2 diploids liquid holding treatment results in the reduction of the maximum yield of recombinants at 200 ergs/mm^2 from 5.4% to 3.2% homozygosis.

Both sets of results indicate that although cell viability remains constant in excision-deficient diploids after liquid holding treatment the levels of both recombination and mutation are modified. The results may be explained on the basis of a model of liquid holding recovery in which mutation and recombination are reduced by the reduction in the activity of a post-replication repair process whose contribution to cell viability is replaced by a repair process acting only in diploid yeast cultures.

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Evans, W.E. and J.M. Parry (1974) Isolation, genetics and survival characteristics of thermo-sensitive mutants of yeast. Mol. Gen. Genetics 134 333-344.

Parry, J.M. (1974) The selective effects of herbicides upon the growth of yeast cultures. Arch. Microbiol. 98 331-338.

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Contract N. 111-72-1 BIOI

Prof. G.E.Magni and Prof.N.Loprieno

MOLECULAR NATURE OF POINT MUTATIONS INDUCED BY X-RADIATIONS

Summary - The objective of the proposed research was to obtain in eucaryotic organisms evidence on a large scale on the distribution of molecular types of point mutations in relation to the effectiveness of repair mechanisms. In Saccharomyces cerevisiae dose/effect curves for the induction of transitions AT⇄GC and transversions in the codon tyr7-1 were obtained both for sensitive cells (lack of repair) and resistant ones where repair mechanisms are fully expressed.

In Schizosaccharomyces pombe the specific mutantion rate per locus per rad in different genetic and phenotypic systems has been evaluated, on producing more extensive experimental evidences in the yeast for the Abrahamson's et al. hypothesis (1973). The specific gene-conversion rate per locus per rad has been evaluated in three different wild type double heterozygotic combinations in the ade7 locus, as a preliminary investigation for a more different study of the role of the repair processes on genetic effects produced by X-rays.

Comparison made on the specific mutation rates induced by chemical mutagens and X-rays show that the former are more efficient : MMS and EMS produce respectively 40 and 10 times more mutations than X-radiations.

Project N.1

Prof.G.E.Magni, ProfG.P.Sironi, Dr.S.Sora,Dr.L.Panzeri

Molecular nature of point mutation induced by X-radiation in Saccharomyces cerevisiae

The aim of research carried out during last year was to obtain complete dose/effect curves (from 20 to 80-100 Krad) for the relative proportions of transitions and transversions induced by X-radiations in synchronized cells of Saccharomyces cerevisiae.

a) The screening system.

The method used is based on the quantitative analysis of the following mutational events occuring in a specific codon of the gene tyr7 (tyrosine and phenylalaine dependence) :

Strain 2a : $tyr7\text{-}1^{o}$(ochre) $\longrightarrow$ $tyr7\text{-}1^{a}$(amber) = transitions AT→GC

$tyr7\text{-}1^{o}$ $\longrightarrow$ sense codons = transitions AT→GC + transversions

Strain 10a : $tyr7\text{-}1^{a}$(amber) $\longrightarrow$ $tyr7\text{-}1^{o}$(ochre) = transitions GC→AT

$tyr7\text{-}1^{a}$ $\longrightarrow$ sense codons = transitions GC→AT + transversions

Full details on the technical procedure will be published soon.

b) Transitions and transversions in synchronized cells.

Cultures of the above strains, synchronized according to the general procedure outlined in the previous report,show the survival curves of Fig.1, they contain 99,3 and 99,6% of sensitive cells.

In fig. 2 the dose/effect curves for the induction of AT →GC transitions and NS sense reversions are indicated for both the X-radiation sensitive and resistant fraction of the cell population. Transitions seems to be induced in much lower proportion than transversions in either the sensitive fraction of cells or the resistant one, where the repair mechanisms are fully expressed. An other interesting indication, that

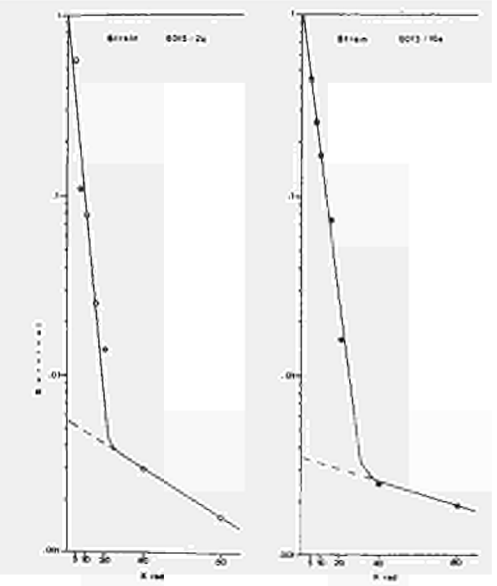

Fig.1

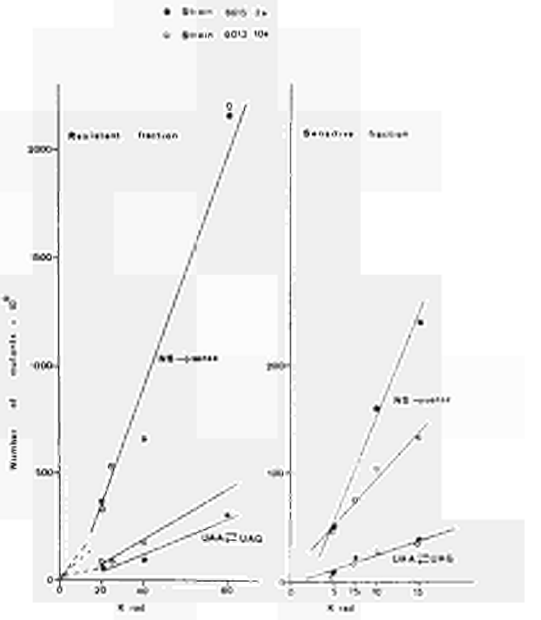

Fig. 2

Fig. 1 Survival curves to X-radiations of strains 2a an 10a

Fig. 2 Induction of UAA $\rightleftarrows$ UAG transitions and of nonsense $\longrightarrow$ sense mutations

must be however more carefully checked, is the apparent higher sensitivity of the "resistant" cells to the induction of both transitions and transversions.

Should further extension of our data confirm this preliminary evidence, we might conclude that repair mechanisms to X-radiation induced damage are essentially "error prone" in Saccharomyces cerevisiae.

Publications :

G.E.Magni, S.Sora, L.Panzeri - Base substitutions induced by X-radiations in synchronized cells of Saccharomyces cerevisiae. In abstracts of V Int.Congress Rad.Res (1974)pg.99.

Project N. 2

Prof. N. Loprieno, Dr. A. Abbondandolo, Dr. R. Barale, Dr. S. Baroncelli and Dr. A.M. Rossi.

Molecular nature of point mutations induced by X-radiations in Schizosaccharomyces pombe.

Progress report

Further studies on the kinetics of mutation induction by X-rays in a repair-deficient strain of S.pombe, in comparison with wild type have been developed, on analyzing forward mutations at five loci.

A specific mutation rate of 0.14×10^{-8} x locus x rad has been evaluated for the wild type, whereas for the repair deficient strain the value found has been of 0.13×10^{-7} x locus x rad. On normalizing these values for the DNA content of haploid nucleus of S.pombe (0.0225 pg = 7.75×10^{-3} human DNA), the specific mutation rate found have been of 1.80×10^{-7} and 1.67×10^{-6} respectively. The wild type's value is very close for that reported for S.cerevisiae in the table presented by Abrahamson et al. (Nature 245 (1973) 460-462), thus indicating a general value of Abrahamson's data, although the repaur ability of the biological material should be taken into account when considering genotype heterogenicity among individual of the same species. The specufic mutation rate however is very strongly influenced by the spontaneous mutation background.

When compared with chemical mutagens. X-rays have been shown to be less efficient for the induction of specific forward mutation rate: in the same repair deficient strain of S.pombe (rad10-198) methyl methanesulfonate (MMS) has shown a mutation rate of 5.02×10^{-7} x locus x uM, equale to

38.6 radequivalent; ethyl methanesulfonate (EMS) has shown a mutation rate of 1.88×10^{-7} x locus x uM, equale to 11.2 rad-equivalent. These chemicals have displayed the same kinetic induction as that found and described for X-rays, thus indicating that in the repair deficient strain the production of mutation is a direct process on the DNA (Loprieno et al., 1975; Loprieno et al., 1974), whereas in the wild type mutations are mainly the results of an error - prone active system.

Analyses done on the molecular nature of mutants induced by X rays indicate that among mutants induced in the wild type 45% of base-pair substitution mutations has been obtained, whereas in the repair deficient strain only 2.2%.

In order to evaluate the role of repair activity in the production of other genetic damages different from mutations, such as gene-conversion, a process which might influence the genetic variability among individuals, the genetic activity of X-rays has been also analyzed for the production of gene-conversions in three diploid combinations (double heterozygotes) of ade7 mutant alleles: all three strains present the same inactivation kinetics, but a specific gene-conversion rate of 0.41×10^{-9}, 2.88×10^{-9}, $5,65 \times 10^{-9}$ x locus x rad respectively for the three diploids (in the three combinations, the ade7 alleles are located at different distances), values very similar to that observed for the specific mutation rate in the wild type (= 1.42×10^{-9} x locus x rad). At the value of 37% survival the following data have been respectively obtained for the production of gene-conversions: 42.10×10^{-6}, 420.81×10^{-6}, 947.82×10^{-6}, whereas at the same survival value an induction of 446.06×10^{-6} mutations per locus was observed.

As a general consideration it may be assumed that in

S.pombe the induction of gene-conversion and mutation has quantitative similar expressions.

Experiments have done with the chemical methyl methanesulfonate (MMS) for a comparison with the X-rays ability to induce gene-conversions. Strains have been synthetized which combine heterozygotic alleles in the ade7 locus and homozigosity for the rad10-198 allele.

The following paper has been accepted for publication in Mutation Res.:

Loprieno, N. et al.: Mutations induced by X-radiations in the yeast S.pombe.

The following papers are scheduled to be published in Mutation Res. during 1975:

1) Abbondandolo, A.: Mutation and nuclear stage in Schizosaccharomyces pombe. I. A genetic system for studying the role of recombinational repair in mutation induction. Mutation Res. 27 (1975).
2) Rainaldi,G. and A. Abbondandolo: Mutation and nuclear stage in Schizosaccharomyces pombe. II. Reverse mutations induced by X rays in the absence of recombination. Mutation Res. 27 (1975).

Contractant de la Commission : Fondation Curie-Institut du Radium

N° du contrat : 126-74-7 BIOF

Chef du (des) groupe(s) de recherche : R. LATARJET, Directeur

Thème général du contrat : Influences des structures particulières des acides nucléiques sur la nature de leur radio-lésions. Conséquences de l'efficacité des processus de réparation et sur le problème de la radioprotection.

Description générale succincte des travaux réalisés :

Il est reconnu que du bactériophage aux cellules de vertébrés supérieurs une fraction des radiolésions est réparable par des processus enzymatiques cellulaires. La fraction résiduellement non réparée des radiolésions et la non fidélité de la restauration sont responsables de la "mort" ou des altérations des fonctions biologiques examinées. Les altérations chimiques produites par les radiations dans les acides nucléiques de bactéries "hyperrésistantes" tel que Micrococcus radiodurans sont quantitativement et qualitativement les mêmes que dans les bactéries de radiosensibilité "normale". De plus, les enzymes de réparation ne sont pas plus abondantes ou différentes dans les deux types de bactéries.

L'association particulière de l'ADN et de la membrane plasmique est sans doute à l'origine de la radiorésistance exceptionnelle de certaines bactéries. C'est la nature de cette association que nous tentons de clarifier dans le premier projet.

Le second consiste à définir dans des cellules eucaryotes (levures et cellules de mammifères en culture) les interelations nucléaires et mitochondriales des processus de réparation de ces deux types d'ADN.

Le troisième projet, de caractère radiobiochimique, consiste à donner un support moléculaire aux recherches précédentes, c'est-à-dire à définir chimiquement les altérations des structures des acides nucléiques.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques : Drs. N. REBEYROTTE, J.P. THIERY et Mlle M. DARDALHON-SAMSONOFF

Titre du projet : Rôle de l'attachement de l'ADN à la membrane dans la réparation des radiolésions chez Micrococcus radiodurans.

Description des résultats :

Le complexe ADN-membrane est isolé après la lyse de Micrococcus radiodurans. Afin de préciser son rôle dans la réparation des lésions induites par les rayons X, l'effet de différents agents tel qu'un inhibiteur de la synthèse protéique (chloramphénicol) ou d'un radiosensibilisateur (l'iodoacétamide) ont été étudiés. Parallèlement des techniques cytochimiques permettant de caractériser les charges négatives, au niveau des ultrastructures cellulaires, par capture de colloïdes métalliques, ont été élaborées.

a) Effet du chloramphénicol : Si Micrococcus radiodurans est irradié à une dose sublétale de 2.10^5 Rads, 90 % de l'ADN sédimente librement dans un gradient de sucrose neutre, 10 % seulement de l'ADN reste lié avec une fraction membranaire. En revanche dans le cas où les bactéries sont traitées au chloramphénicol avant l'irradiation 35 % de l'ADN est fixé sous forme de complexe. L'incubation de post-irradiation en milieu complet additionné de chloramphénicol ne modifie pas la proportion d'ADN libre. Cette association ADN-membrane est détruite si l'incubation de post-irradiation est effectuée en absence de chloramphénicol. La cinétique de cette dissociation a été établie ; l'effet du chloramphénicol dans le milieu de post-irradiation est en effet réversible par lavage et resuspension des bactéries dans un milieu complet. L'ADN libéré présente des ruptures double chaine. Les protéines nouvellement synthétisées après un traitement par les radiations ionisantes interviennent dans la formation de cassures dans la molécule d'ADN entrainant sa libération du complexe ADN-membrane. Ces ruptures semblent jouer un rôle essentiel dans l'étape de réparation de l'ADN qui se ferait par l'intermédiaire du complexe.

b) Effet de l'iodoacétamide : L'iodoacétamide sensibilise Micrococcus radiodurans à l'effet létal des radiations ionisantes. Son action sur le complexe ADN-membrane a été étudiée. On obtient les résultats suivants :

- pour les bactéries non irradiées, l'iodoacétamide ne modifie pas les complexes ;
- pour les bactéries irradiées en présence d'iodoacétamide (10^{-3} M) les profils de sédimentation sont les mêmes que ceux obtenus sans l'agent chimique ;
- pour les bactéries irradiées en présence d'iodoacétamide, puis incubées en milieu de croissance, on n'observe plus la réassociation de l'ADN à la membrane caractéristique des bactéries irradiées en l'absence de ce composé.

Le rôle radiosensibilisateur de l'iodoacétamide s'expliquerait donc par une inhibition de cette étape de la réparation de l'ADN par cet agent chimique.

L'ensemble de ces résultats montre que la réassociation de l'ADN à la membrane pendant l'incubation de post-irradiation intervient dans la réparation des radiolésions impliquant plus particulièrement la restauration des ruptures double chaine. La synthèse de nouvelles protéines est nécessaire à cette étape.

Publications

1) Rôle de l'attachement de l'ADN à la membrane dans la réparation des radiolésions chez Micrococcus radiodurans. M. DARDALHON-SAMSONOFF et N. REBEYROTTE. Int. J. Rad. Biol. (sous presse).
2) Cytochimie sur coupe fine des polysaccharides acides. J.F. THIERY. J. Microscopie, 1974, 20, 94a.

The non-irradiated bacterial DNA associated to the membrane is liberated into the cytoplasm, after breaking of either a single or a double strand, resulting from X ray action.

During the reincubation period in growth medium the DNA is reassociated to the membrane.

This phenomenon is very rapid and occurs without increasing the molecular weight of DNA. The study of DNA-membrane complexes shows that the size of the DNA-associated membranous fragment differs according to the lysing technique employed, which appears as a change in the density of the complex.

Chloramphenicol decreases reassociation, and iodoacetamide, a radiosensitising agent, inhibits it completely.

Résultats du projet n° 2

Chef de projet et collaborateurs scientifiques : Drs. E. MOUSTACCHI, P. JULLIEN, F. FABRE, R. CHANET, M. HEUDE et R. WATERS

Titre du projet : Réparation des radiolésions des ADN nucléaires et mitochondriaux dans des cellules eucaryotes.

Description des résultats :

1) Excision des dimères de pyrimidine (pyr[ ]pyr) dans les ADN nucléaires et mitochondriaux chez la levure.

a) La proportion de pyr[ ]pyr induits par les UV est la même dans des cellules haploides (n) et diploides (2n). L'excision de ces dimères est également la même dans différentes conditions de post-irradiation. Autrement dit, la différence entre cellules n et 2n de radiorésistance et de réparabilité de la survie en étalement différé (liquid holding recovery) ne sont attribuables ni à une différence dans la quantité de ces radiolésions, ni à celle de l'aptitude à les exciser pour les deux degrés de ploidie. Il est vraisemblable que des différences dans la capacité à recombiner le matériel génétique soient en cause.

b) L'étude de l'excision des pyr[ ]pyr en fonction de la dose d'UV met en évidence un phénomène de saturation : dans la région de dose qui correspond à l'épaulement des courbes de survie jusqu'à 70 % des pyr[ ]pyr induits sont excisés, aux doses plus fortes, on assiste à une diminution progressive de ce pourcentage. Ce phénomène de saturation du processus d'excision a été décrit également pour les cellules de mammifères irradiées en culture. Il implique une inactivation de cette fonction par des fortes doses d'UV.

c) Après une irradiation, l'excision des pyr[ ]pyr se produit si les cellules sont incubées en milieu complet ou en solution saline. Elle est augmentée dans les conditions qui miment celles de la réparation de la survie ou liquid holding recovery, c'est-à-dire lorsque les cellules sont incubées d'abord en saline puis en milieu complet. Ces résultats démontrent l'importance du processus d'excision dans la restauration de la survie.

d) L'excision ne se produit pas en saline chez un mutant cytoplasmique déficient respiratoire (ρ^-), elle est faible en milieu complet par rapport au type sauvage (ρ^+) et n'est pas améliorée par la double incubation

en saline puis en milieu complet. Ceci montre qu'un système respiratoire fonctionnel contribue à l'efficacité du processus d'excision. Il faut noter une corrélation entre cette défectivité dans l'excision de ce mutant ρ^- et sa plus grande radiosensibilité ainsi que son inaptitude à subir une réparation en "liquid holding".

e) Pour des cellules en phase de croissance stationnaire l'ADN mitochondrial de cellules irradiées est rapidement dégradé dans toutes les conditions d'incubation examinées. Le pourcentage de pyr[ ]pyr qui reste dans la fraction non encore dégradée de l'ADN mitochondrial est constante, et égale à celle des échantillons irradiés et examinés immédiatement après l'irradiation. Autrement dit, dans ces conditions, il n'apparait pas qu'un système d'excision contrôlé agisse au niveau de l'ADN mitochondrial. Là encore une corrélation apparait entre le liquid holding négatif observé pour l'induction de ρ^- par les UV et cette dégradation intensive de l'ADN mitochondrial.

La photoréactivation des pyr[ ]pyr induits dans l'ADN mitochondrial a lieu dans les mêmes conditions que pour l'ADN nucléaire. Ces radiolésions sont donc reconnues de la même manière par l'enzyme de photoréactivation au niveau des deux localisations possibles des dimères (noyaux et mitochondries).

2) Effet des rayons X sur la capacité des cellules de mammifères à former des hybrides viables.

L'étude des effets des rayons X sur la capacité des cellules somatiques à former des hybrides par fusion avec un partenaire non irradié a été poursuivie. Cette étude porte sur des lignées de cellules mutantes, qui sont déficientes soit en hypoxanthine-guanine-phosphoribosyl-transferase (lignée A9, dérivée des cellules L de souris), soit en thymidine kinase (lignée C1ID, dérivée des cellules L de souris, et lignée B1 dérivée des cellules BHK21 de hamster) ; ces trois lignées sont incapables de croître en milieu sélectif HAT (hypoxanthine-amethopterine-thymidine) alors que les hybrides dérivés de la fusion cellules A9-cellules C1ID (croisement intraspécifique) ou de la fusion cellules A9-cellules B1 (croisement interspécifique) donnent naissance à des colonies dans ce milieu sélectif HAT.

L'irradiation d'une lignée parentale préalablement à sa fusion avec un parent non irradié diminue le nombre d'hybrides capables de se révéler par la formation d'une colonie en milieu sélectif. L'aspect des courbes de la réduction du nombre d'hybrides en fonction de la dose d'irradiation

varie selon la lignée cellulaire irradiée.

Seule la lignée B1 (cellules de hamster) présente une décroissance exponentielle continue de sa capacité d'hybridation avec des cellules A9 pour des doses allant jusqu'à 4000 R ; la Do est de l'ordre de 750 R. Les deux lignées de cellules de souris présentent des courbes diphasiques : de 0 à 2000 R la décroissance du nombre d'hybrides est exponentielle, après une légère épaule (Do des cellules C1ID = 500 R, Do des cellules A9 = 300 R), puis les courbes cassent et s'incurvent vers le haut. En ce qui concerne les cellules A9, on note même un plateau de radiorésistance dans l'intervalle de dose de 2000 à 4000 R. Il est à noter que la radiosensibilité de la capacité d'hybridation des cellules A9 est pratiquement la même lorsqu'elles sont fusionnées soit avec des cellules C1ID, soit avec des cellules B1.

En dépit des différences des effets des rayons X sur la capacité d'hybridation en fonction de la lignée cellulaire, il a été possible avec les trois lignées utilisées d'obtenir des hybrides avec un nombre de cellules exposées à une dose de rayons X suffisante pour leur faire perdre leur capacité de donner naissance à une colonie de façon autonome en milieu non sélectif ($7,5 \times 10^5$ cellules exposées à 3000 et 4000 R). Ces colonies dérivées d'un parent irradié avec des doses relativement élevées apparaissent tardivement (3 semaines après la fusion) et ont une croissance lente. Après quelques semaines elles peuvent cependant être repiquées et finalement donnent naissance à des cellules se multipliant rapidement. Des cellules dérivées de colonies obtenues par fusion de cellules A9 irradiées avec des cellules B1, ou de cellules B1 irradiées avec des cellules A9, portent à leur surface des antigènes d'espèce de l'un et l'autre parent. Ce résultat suggère fortement que le partenaire irradié contribue à la formation d'un hybride viable non seulement en apportant l'information génétique pour la synthèse de l'enzyme manquant au partenaire non irradié, mais aussi en fournissant d'autres informations génétiques qui sont répliquées et exprimées au cours de générations cellulaires successives.

Publications

- The fate of UV-induced pyrimidine dimers in the mitochondrial DNA of Saccharomyces cerevisiae following various post-irradiation cell treatments. R. WATERS et E. MOUSTACCHI. Biochim. Biophys. Acta, 1974, 366, 241-250.
- The dose-dependence of the excision of UV-induced pyrimidine dimers from the nuclear DNA's of haploid and diploid Saccharomyces cerevisiae. R.

WATERS et E. MOUSTACCHI. J. Bacteriol., 1974 (accepté et sous presse).
- Protein synthesis and the recovery of both survival and cytoplasmic "petite" mutation in UV treated yeast cells. I. Nuclear directed protein synthesis. M. HEUDE, R. CHANET et E. MOUSTACCHI. Mutation Res., 1974 (accepté et sous presse).
- Protein synthesis and the recovery of both survival and cytoplasmic "petite" mutation in UV treated yeast cells. II. Mitochondrial protein synthesis. M. HEUDE et R. CHANET. Mutation Res., 1974 (accepté et sous presse).
- The present status of DNA repair mechanisms in UV irradiated yeast taken as a model eucaryotic system. E. MOUSTACCHI, R. WATERS, M. HEUDE et R. CHANET. Radiation Res., Supplement 1974, Proc. of the Vth Int. Congress of Radiation Research, 20 pages (sous presse).

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1) The induction and excision of UV-induced pyrimidine dimers from the yeast nuclear and mitochondrial DNA was studied as a function of UV dose, according to ploidy, the ρ^+ or ρ^- (mit. DNA_o) condition of the strains and various physiological parameters.

A saturation of the excision-system is found at relatively low doses. A similar pattern of induction and removal of dimers is seen for both haploid and diploid cells. An intact mitochondrial genome is required for an optimal excision efficiency. Finally an extensive degradation of the mit. DNA is primarly seen in UV irradiated stationary phase cells.

2) The effect of X-rays on the capacity of mammalian cells to form a viable hybrid was studied. The genetic systems are described. The irradiated partner contributes in the formation of a viable hybrid a) by bringing the genetic information for the synthesis of the specific enzyme lacking in the unirradiated partner ; b) by furnishing other genetic informations which are replicated and expressed in the successive cellular generations.

Résultats du projet n° 3

Chef du projet et collaborateurs scientifiques : Drs. B. EKERT, F. POCHON et Mme N. GIOCANTI

Titre du projet : Bilan radiochimique des lésions des divers complexes nucléiques.

Description des résultats :

Les interactions entre un acide nucléique (ARN ribosomal) et une protéine particulière du point de vue radiobiologique ont été étudiées sur le système in vitro suivant : un inhibiteur naturel, fixé sur les ribosomes d' Escherichia coli, qui freine la biosynthèse des protéines a été précédemment identifié par nous ; en fonction des conditions expérimentales cet inhibiteur peut être fixé ou détaché des ribosomes. Dans un premier temps la sensibilité de cet inhibiteur aux rayons γ a été définie puis les courbes d'inactivation des ribosomes avec l'inhibiteur fixé ou non ont été établies. On montre notamment que la présence de ce produit modifie considérablement l'allure exponentielle de la courbe d'inactivation des ribosomes exempts de l'inhibiteur.

L'irradiation γ du complexe ribosomes-inhibiteur stimule l'activité ribosomale en détruisant progressivement l'inhibiteur au niveau de ces particules que l'inhibiteur soit à l'état natif sur les ribosomes ou qu'il soit ajouté et refixé in vitro à des ribosomes purifiés.

L'isolement et la purification par chromatographie sur gel de l'inhibiteur a été accomplie. Sa localisation par recombinaison croisée de particules 50 s et 30 s a été précisée. Il s'avère que ce composé est fixé aux deux types de particules, les fractions 30 s se révèlent cependant plus actives. Les propriétés et le mode d'action de cet inhibiteur ont été définis.

Publication

Fluorescent labels on ribosomes. The interactions of rRNA and proteins. F. POCHON, B. EKERT and M. PERRIN. Eur. J. Biochem. 1974, 43, 115-124.

The interactions between ribosomal RNA and a bound natural inhibitor of the protein biosynthesis were studied with respect of the effects of γ-rays.

A stimulation of the ribosomal activity is found when this complex is irradiated. A progressive destruction of the inhibitor is responsible for this effect. The inhibitor has been isolated and characterised.

Contractor:	National Radiological Protection Board
Contract No.:	131-74-1 BIO UK
Head of research team(s):	Dr. G. W. Dolphin
General Subject of Contract:	Radiation-induced chromosome aberrations

Two factors which affect the yield of radiation-induced chromosome aberrations in human lymphocytes are studied in the two projects under this Contract. In the first project, two different techniques are used to investigate the effects of dose rate on aberration yield; split dose radiation, in which two equal doses are separated by various time intervals, and continuous radiation using several dose rates. In the other project the effect of LET on aberration yield is being examined. Work in the past year has been concentrated on the development of necessary instrumentation for the dosimetry of the proposed neutron irradiations, and the development of computer programs designed to enable the results of biological experiments to be interpreted in terms of microdosimetry.

Results of Project No.: 1

Head of Project and Scientific Staff: D. C. Lloyd
R. J. Purrott

Title of Project: The effect of dose rate on the yield of radiation-induced chromosome aberrations

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Two experimental procedures have been used to study the effect of dose rate on chromosome aberration yield in human peripheral blood lymphocytes. In both experiments enriched lymphocyte mini-cultures were prepared and incubated for 48 hours. They were then analysed for unstable chromosome aberration frequency.

Experiment A. Samples of unstimulated human peripheral blood were exposed at 37°C to doses of 500 or 200 rads of 250 kV X-rays at 100 rads per minute. Each dose was split into two equal fractions of 250 or 100 rads separated by intervals of 0.25 to 7.5 hr. The results are shown in figure 1. For both doses the yield of dicentrics fell as the time between fractions was increased. The dotted reference lines for 500 and 200 rads single exposures were obtained from X-ray dose response data previously published in reports from this laboratory. Base lines were calculated on the assumption that no interaction takes place between the damage induced by the two fractions and are thus twice the yields for the half-doses.

Experiment B. 100 and 250 rad doses of caesium-137 gamma radiation were given to samples of unstimulated whole blood at 37°C. Dose rates ranged from 5 to 400 rads per hour. In figure 2 the dicentric yields for 100 and 250 rads have been plotted against the duration of the exposure.

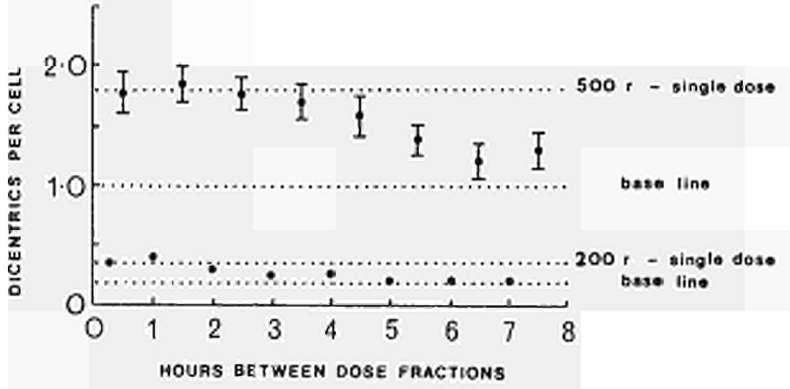

Figure 1: Variation of dicentric yield following X-irradiation with two fractions of 100 or 250 rads separated by intervals of 0.25 to 7.5 hours

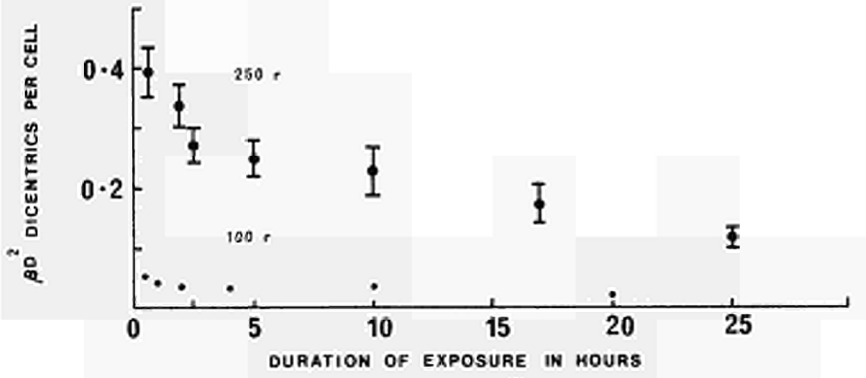

Figure 2: Variation of the βD^2 contribution to dicentric yield plotted against the time taken to deliver 100 or 250 rad doses of Cs-137 gamma rays

In the NRPB laboratory it has been found that the best fit of the dicentric chromosome aberration yield (Y) to radiation dose (D) is obtained with the quadratic function: $Y = \alpha D + \beta D$, where α and β are constants. A physical interpretation of this function is that the linear dose term represents dicentrics produced by a single track and the squared term dicentrics produced by two separate tracks. These two tracks may not be simultaneous and repair mechanisms may intervene and anneal the first lesion so that it cannot react with the second to form a dicentric. Thus by reducing dose rate or by fractionating exposures the yield from the βD^2 term will be decreased.

Figure 1 shows that by 7.5 hr the base line of the fractionation effect has not yet been reached for the split 500 rad exposures. Using a value for the αD term obtained in this laboratory it can be shown that the lesions induced by this dose have a mean-life of about 8 hr. The 200 rad data appear to be additive by intervals of only 5 hr. This is therefore an indication that the rate at which lesions are annealed may be dose dependent.

Dicentric yields plotted in figure 2 represent only the βD^2 contribution; the αD component, which by definition does not depend on dose rate, has been removed. For the data at 250 rads the yields show a marked decline from 0.4 to 0.25 dicentrics/cell when the dose is spread over 5 hr and this is consistent with the estimate of an 8 hr mean-life of a break.

For the future the 500 rad fractionation experiment will be extended using intervals of 8, 16 and 24 hr. In the variable dose rate study additional experiments are planned in which the duration of the exposure will be prolonged to 50 hr for all doses including an additional series at 500 rads.

Results of Project No.: 2

Head of Project and Scientific Staff: J. A. Dennis
A. G. Sherwin
D. G. Jones
A. A. Edwards
B. L. Davies
M. J. Whillock

Title of Project: The dependence of radiation-induced chromosome aberrations on radiation quality

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A neutron spectrometer based on an organic scintillator has been developed and used in attempts to measure neutron spectra above 2 MeV. These attempts revealed discrepancies in the results that are attributed to inaccurate cross-section data. This problem is being studied further.

Two ionization chambers, each of nominal capacity 1cc, have been constructed, with graphite and CH-plastic walls respectively. These will be used to determine the gamma and neutron components of mixed field conditions using a new method of interpretation based on an analysis of the stochastic variations in the observed currents from each chamber.

Publications:

(a) Developments in the use of ionization chambers for mixed field dosimetry. A. G. Sherwin. Proc. 2nd Symp. on Neutron Dosimetry in Biology & Medicine. 1974

(b) Some qualitative considerations affecting the variation of W-values. J. A. Dennis. Proc. 2nd Symp. on Neutron Dosimetry in Biology & Medicine. 1974

Dose-effect relationships for chromosome aberrations usually take the form: $A = \alpha D + \beta D^2$ .....(1)

A is the observed frequency of aberrations
D is the absorbed dose
α and β are coefficients.

Such relationships may be regarded as the low dose form of the more generally fitted multi-target dose-effect relationship:

$$A = (1-\exp D/D_o)^n \quad \text{.....(2)}$$

Other models of dose-effect relationships are gaining in popularity. Most, however, lead to a similar interpretation of the low dose forms, which is that the observation of a real first coefficient, α, in equation (1) suggests the existence of a single hit single target mechanism, but a positive second coefficient, β, implies that either a double-hit or a double target mechanism is possible also.

The value of D_o in equation (2) or $1/\alpha$ in equation (1) can be taken as that dose at which an average of one hit has occurred in a sensitive target volume of the cell. Naturally, since the distribution of hits is a stochastic process some volumes will have received more than one hit and some targets will have received no hits. If a hit is defined as the penetration into or production of an ionizing particle within the target volume, then simple physical considerations, i.e. the fluence of secondary particles produced within irradiated tissue, imply for D_o values for neutrons in the range 100-300 rads (α in the range 0.01 to 0.003 rad^{-1}) that the target volumes must have minimum diameters of about 1 μm. Any other definition of a hit, e.g. that a certain minimum energy must be deposited in the target volume or the effectiveness of a hit depends on the energy deposited within the target, would lead to an increase in this minimum diameter. It should be noted, however, that it is not possible to explain observed variations of RBE with neutron energy if the simple definition of a hit used above is adopted. This implies firstly that the various secondary particles produced in tissue by neutrons differ in their biological efficiency and secondly that the target volumes must exceed the minimum value of 1 μm.

Publications:

The calculation of fluence, LET and event size spectra for neutron beams. A. A. Edwards. Proc. 2nd Symp. on Neutron Dosimetry in Biology and Medicine. 1974.

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

Contract No.: 134-74-1 BIOUK

Head of research team: D.H. Peirson

General subject of contract: MICRODOSIMETRY STUDIES

The aim of this research is to provide microdosimetric models for the interaction of radiations of a range of LET's with mammalian cells and so improve the basis from which quality factors used in radiological protection are derived. The contract is divided into two projects with the initial objective of supplying biological data for the models.

Project 1. The mutagenic and lethal effects of neutrons on Chinese hamster cells

A strain of Chinese hamster cells, V79-4 has already been established and we have examined the properties of a somatic cell mutation assay which is that of resistance to the purine analogue 8-Azaguanine. This resistance is due to the loss of one of the enzymes involved in the utilization of purines. The survival and mutation responses of the cells is being studied for a range of neutron spectra with mean energies from 1 to 14 MeV.

Project 2. Development of a mouse lymphoma mutation assay system

The mouse lymphoma L5178Yd has been established in our laboratory and is grown in suspension. The mutation assay system uses a soft agar cloning technique and up to six loci can be examined with mutation rates varying from 2×10^{-5} to 1×10^{-7}. This system should provide an independent test of the models devised for the effects in Chinese hamster cells.

Results of Project No. 1

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

Title of Project: THE MUTAGENIC AND LETHAL EFFECTS OF NEUTRONS ON CHINESE HAMSTER CELLS

Previously we had measured the survival and mutation response of the cells to gamma radiation and to neutron spectra with five different mean energies. These measurements showed that the mutation response to neutrons was linear, unlike that to gamma rays. This would imply that the relative biological effectivess (RBE) of neutrons compared with gamma rays increased indefinitely as the dose was reduced.

In the period of this report we have measured the response of the system to an additional neutron quality and have also studied the mutation response to lower doses of gamma radiation. This latter experiment has shown that there is a linear component in the gamma ray response and hence the RBE for neutrons does not increase indefinitely as the dose is reduced but has a limiting value of about 4.

We have also measured the effect of fractionating the gamma ray dose on mutation induction and survival. When a single dose of 1260 rads which corresponds to a survival of 0.7% is split into two equal fractions separated by 6 hours, then the induction of mutations is reduced by a factor of 10. This is being studied further.

Results of Project No. 2

Head of Project and scientific staff: J.C. Asquith
P.D. Holt

Title of Project: DEVELOPMENT OF A MOUSE LYMPHOMA MUTATION ASSAY SYSTEM

The mouse lymphoma cells L5178Yd which were obtained from Dr C F Arlett* are routinely subcultured in our laboratory. However, experimental work this year has been restricted to determining the best conditions and techniques for obtaining reproducible plating efficiency. A 1.15% sodium pyruate addition to the growth medium is important. In plating experiments, dry boxing, quick gassing and rapid cooling after the addition of noble agar are desirable.

Preliminary investigations of the survival curve are now being carried out.

* Medical Research Council, Cell Mutation Unit, University of Sussex

Associato della Commissione: Università di Pavia
Gruppo Euratom - Istituto di Biologia Generale. Facoltà di Medicina.
N° del contratto: 112-72-1 BIOI
Capo del gruppo di ricerca: Prof. M. Fraccaro

Tema generale del contratto: Localizzazione dell'effetto della radiazione interna da precursori tritiati delle basi del DNA nei cromosomi umani.

Breve descrizione generale dei lavori compiuti.

Sono state allestite normali colture di linfociti da sangue periferico. A 64 h di incubazione ciascuna coltura è stata sdoppiata in due colture parallele. Dopo due ore alle due colture parallele sono state aggiunte quantità equivalenti di desossicitidina-5-H^3 (^{3}H-DC) e di timidina-(-2-deossi-D-ribosio-5-H^3), alla stessa attività specifica. Colchicina è stata aggiunta 4 ore prima della raccolta delle cellule. Dopo uno screening su vetrini colorati con orceina acetica per conoscere il numero e il tipo di lesioni cromosomiche, altri vetrini venivano colorati con mostarda di chinacrina per localizzare i punti di rottura nei cromosomi.

Descrizione dei risultati

Nei vetrini di colture marcate con 10μCi/ml di ^{3}H-DC, il 37.5% delle cellule presenta aberrazioni cromosomiche con una media di 0.44 lesioni/cellula. Nei vetrini allestiti dalla coltura parallela marcata con 10μCi/ml di ^{3}H-TdR, le cellule con aberrazioni cromosomiche rappresentano il 33.7% con 0.36 lesioni/cellula.

In colture marcate con 5 μCi/ml rispettivamente di ^{3}H-DC e ^{3}H-TdR il numero di cellule con rotture diminuisce al 29.5% in colture marcate con ^{3}H-DC (0.37 lesioni/cellula) e al 28.2% in colture marcate con ^{3}H-TdR (0.30 lesioni/cellula). in tutte, le lesioni acromatiche sono le più frequenti, seguite da rotture cromosomiche e i frammenti. Più rari i riarrangiamenti (3%).

In seguito a colorazione per le bande R si è notato che i punti di rottura sono selettivamente situati sulle bande non fluorescenti. In conclusione, non sembra che a somministrazioni di basi radioattive diverse corrispondano diversità nel numero e nel tipo di rotture e tentomeno nella localizzazione dei punti di rottura sui cromosomi. Infatti tali rotture sono comunque situate selettivamente sulle bande non fluorescenti. Se ne deduce perciò che la chinacrina non si lega a regioni del DNA caratterizzate da alti rapporti AT/GC nè che le zone intensamente fluorescenti siano quelle ad alto contenuto in A-T. I dati riportati portano invece a ritenere le proteine o meglio il loro modo di porsi lungo la molecola del DNA, responsabile del manifestarsi delle bande. Le stesse proteine potrebbero poi - a secondo di come si legano al DNA - formare una protezione dal danno delle basi radioattive del DNA.

Contractant de la Commission : Institut National de la Recherche Agronomique

Nº du contrat : 097-72-1 BIO F

Chef des groupes de recherche : Marc A. DALEBROUX, Fonctionnaire scientifique de la Commission

Thème général du contrat :

Etude des effets génétiques, aux plans population et cellulaire, des rayonnements ionisants :

I. Effets des radiations ionisantes sur un caractère de fitness important chez Habrobracon juglandis.

II. Réactions génétiques cellulaires aux rayonnements ionisants chez Nicotiana tabacum.

PROJET Nº I

Chef du Projet : Marc A. DALEBROUX

EFFECTS OF IONIZING RADIATIONS ON A FITNESS CHARACTER OF HABROBRACON JUGLANDIS

As pointed out in the 1972 Report, it was necessary to investigate the instability of the control. Four lines, called A, B, C and D, are being maintained by continuous full-sibbing in order to increase their inbreeding coefficient. Another line, FS, has been long maintained by full-sibbing. Wasps have also been maintained under two other conditions : (i) one consists of keeping 400 females and 400 males in a population jar every generation ; this is assumed to be the F_0 population, and (ii) one "line" is being kept under drift conditions with a sample size of 20 couples. The drift procedure was started after the line had been submitted to full-sibbing for ten generations. This "line" has been called F_{10}-drift.

One experiment consists of comparing the egg-laying abilities over 25 days, on ten families of six females each, of the following genetic classes : F_0, F_{10}-drift, FS, A, A x B, C x D, and (AxB)x(CxD).

The criteria used are :

- Variance among families within genetic classes
  - i. for the total egg-laying of a family of six females
  - ii. for the mean daily individual egg-laying per family

- Variance within families within genetic classes
  - i. for the total egg-laying per individual
  - ii. for the mean daily egg-laying per individual.

The first experiment was started at the beginning of 1974. Up to now, four experiments have been completed. This is not enough to allow valuable conclusions, and, therefore, a detailed report on this investigation will be written only at the end of the present year.

PROJET N° II

Chef du projet : Hubert DULIEU, Chargé de Recherche au C.N.R.S.

Marc A. DALEBROUX, Fonctionnaire Scientifique de la Commission

CELLULAR GENETIC EFFECTS OF IONIZING RADIATIONS IN NICOTIANA TABACUM

II.1. Least Squares Estimation of Genic Effects at a 2-allele Locus of Nicotiana tabacum

The theoretical basis of this research has been presented in the previous report (Annual Report 1973, 298-300). The system studied was the $a_1^+ - a_1$ locus, involved in chloroplast differentiation (Dulieu, 1974, Mutation Research 25 : 289-304). A complete account of the work has been published (Dalebroux and Dulieu, 1974, Genetica 45 : 61-70). The locus was considered to be in Hardy-Weinberg equilibrium in a population from which a random sample of size n = 16 was drawn. The three genotypes were quantitatively expressed in weight of chlorophyll a and b per unit of leaf fresh weight (μg/g). The population was an F_2 from a_1^+/a_1, thus with gene frequencies equal to 1/2 at the locus under consideration.

Heritabilities in the narrow (N) and broad (B) senses have been estimated :

chlorophyll a :	$\hat{h}^2_N$ = 0.82	$\hat{h}^2_B$ = 0.97
chlorophyll b :	= 0.94	0.94
(a + b) :	= 0.90	0.97
a / b :	= 0.00	0.89

For chlorophyll a , it can be stated that, whereas most of the observed variability in the sample was due to the total genetic effects, the majority of the genetic variability was of additive type, although the dominance component was not negligible. As far as chlorophyll b is

concerned, the same statement as above can be made regarding the part taken in the observed variability by the genetic components. In this case, however, no statistically significant dominance effect could be detected, while considerable genetic variability of additive type was shown to exist. The characteristics of the individual "chlorophyll *a*" and "chlorophyll *b*" effects were found combined in the characteristics of "chlorophyll (*a* + *b*)" and "*a*/*b*" : the additive heritability ($\hat{h}^2_N$) for the total chlorophyll was found to be intermediate between that of *a* and *b* (of course, this result is to be expected since the effects are observed separately) ; as for the *a*/*b* ratio, $\hat{h}^2_N$ was statistically absent, the genetic variability being of dominant type only. This shows for the character (i) a very strong overdominance, (ii) that the values of the two homozygotes are statistically equal, and (iii) that, if it is assumed that chlorophyll *b* originates from chlorophyll *a* (Akoyunoglou *et al.*, 1967, Le Chloroplaste, Ed. C. Sironval, Masson & Cie : 91-98), then one has to admit that the transformation from *a* to *b* is much less efficient in the heterozygote than in both homozygotes.

II.2. Least Squares Estimation of Genic Effects at Two Independent 2-allele Loci of *Nicotiana tabacum*

The theory underlying this work has also been presented in the 1973 Annual Report (pp. 300-303). The system studied was $a_1^+ - a_1$ / $a_2^+ - a_2$ (Dulieu, Mutation Research *25* : 289-304). A population was constructed that mimicked an F_2 from the double heterozygote, thus with gene frequencies equal to 1/2 at both loci. These loci were considered to be in Hardy-Weinberg equilibrium. As in Experiment II.1., the genotypes were quantitatively expressed in weight of chlorophyll *a* and *b* per unit of leaf fresh weight (µg/g). The plants in this experiment were grown *in vitro*, while those in Experiment II.1. were not. Thus, the experimental conditions were not the same . A second experiment involving two loci, performed in conditions similar to those of Experiment II.1., has been undertaken. However, the data are not yet available.

Since genotype a_1/a_1 a_2/a_2 produces pure albino individuals, the a/b ratio was not studied, as 0/0 is a mathematical indetermination. The analysis of variance showed that the estimated additive variability component for locus $a_1^+ - a_1$ ($\hat{A}_{a1}$) has an enormous importance as compared to the dominance component, $\hat{D}_{a1}$, although both are highly significant. However, since the epistatic variability components $\hat{AA}_{a1a2}$ and $\hat{AD}_{a1a2}$ are significant, it is necessary to analyze the average effect of gene substitution at locus $a_1^+ - a_1$, $\hat{a}$, for all three genotypic states of locus $a_2^+ - a_2$. In the presence of a_2^+/a_2^+, this effect is significantly smaller than that observed in the presence of either a_2^+/a_2 or a_2/a_2. It seems that this sort of gene action has some kinship with the type of compensation proposed by Schwartz in maize (1971, Genetics 67 : 411-425) and drosophila (1973, Genetics 75 : 639-641), as well as by Freeling and Schwartz in maize (1973, Biochemical Genetics 8 : 27-36) : In the presence of a_2^+/a_2^+, complete dominance of a_1^+ over a_1 is observed, contrary to what takes place in the presence of a_2^+/a_2 or a_2/a_2, where a_1 becomes dominant over a_1^+, and more so in the presence of a_2/a_2. Thus, the antagonistic action of a_1 against a_1^+ (Dulieu, 1974, Mutation Research 25 : 289-304) increases as the dose of a_2^+ decreases. Therefore, it is not unreasonable to believe that a_1^+, a_1 and a_2^+ compete for the same essential limiting factor. This would strongly support the hypothesis of homeology between the two loci (Dulieu, 1974, Mutation Research 25 : 289-304).

The average effect of gene substitution at locus $a_2^+ - a_2$, $\hat{b}$, is largest in the presence of a_1^+/a_1, practically zero in the presence of a_1^+/a_1^+, but still significant in the presence of a_1/a_1 (except for chlorophyll b). This tends to stress (i) the importance of locus $a_2^+ - a_2$ when allele a_1 is present, and (ii) the enormous help provided by a_1^+/a_1 to effect $\hat{b}$. This type of response again supports the hypothesis that a_1 antagonizes a_1^+, since $\hat{b}$ is zero in the presence of a_1^+/a_1^+ but large as soon as a_1^+ is associated with a_1. Furthermore, it seems that a_1 antagonizes a_2^+ also since, in the presence of a_1/a_1,

$\hat{b}$ is strikingly decreased, even becoming zero in the case of chlorophyll b. This could be explained as follows : in a_1/a_1 , the antagonizing effect of a_1 does not act against a_1^+ absent, but against a_2^+. This, of course, again supports the hypothesis of homeology.

Finally, the average effect of gene substitution at locus $a_1^+ - a_1$ in the presence of a_2^+/a_2 , $\hat{a}(a_2^+/a_2)$, is larger than the average effect of gene substitution at locus $a_2^+ - a_2$ in the presence of a_1^+/a_1, $\hat{b}(a_1^+/a_1)$. This suggests that a_1 has an antagonistic action, in that it competes for the essential limiting factor, while a_2 does not. It was also observed that $\hat{b}(a_1^+/a_1^+)$ is practically zero, while $\hat{a}(a_2^+/a_2^+)$ is highly significant. This might mean that the evantual essential limiting factor has more affinity for locus $a_1^+ - a_1$ than for the other locus.

II.3. Spontaneous and Induced Reversion Rates in a Double Heterozygous Mutant of Nicotiana tabacum var. Xanthi NC. Dose-Response Relationship

Investigation of the dose-response relationship in the lower-dose range is usually limited by the sample size necessary to detect low-frequency events. Efforts have been made in this field of research, for the knowledge of the dose-effect relationship in eucaryotes is of utmost importance for evaluating the radiation hazards in human populations. Several systems have been used in higher plants, as the waxy mutants in barley and maize pollen (Eriksson, 1962, Radiation Botany 2 : 35-39 ; Ehrenberg and Eriksson, 1964, Mutation Research 1 : 139-145 ; Ehrenberg and Eriksson, 1966, Acta Radiol. Suppl. 254 : 73-81 ; Eriksson, 1971, Hereditas 68 : 101-114), the v_{s3}/v_s R/r system in tobacco (Mericle and Mericle, 1965, Radiation Botany 5 : 475-492 ; Sand and Smith, 1968, Genetics 58 : 607-624 ; Sand, 1972, Proc. Nat. Acad. Sci. 69 : 2229-2232) and the pink mutations in Tradescantia stamen (Sparrow et al., 1972, Science 176 : 916-918).

The ability of the double heterozygous mutant a_1^+/a_1 a_2^+/a_2 (greenish-yellow) to phenotypically revert towards green was used to quantify the dose-response relationship at 0, 8, 16, 32, 64, 128 and 256 R of gamma-rays given by a ^{60}Co source with an intensity of 380 R/h.

All the research undertaken up to now on the genetic nature of the events responsible for the phenotypic reversion suggests that, in most cases, the changes take place at locus $a_1^+ - a_1$.

In order to estimate the rate of spontaneous reversion per cell, it was decided to observe palisade cells, as it is known that the growth of this tissue is of monolayer type. It was shown that the probability of reversion remained constant throughout the palisade development and that there was no selection against either cell type (original greenish-yellow and reverted). Under such conditions, the rate p of spontaneous reversion was estimated as follows :

(i) the number of palisade cells per mm^2 was determined on the basis of 200 microscope fields, after sucrose infiltration
(ii) 80 discs, 20 mm in diameter, were punched at random on two plants, and the total number of cells (N) calculated
(iii) assuming that all cells originated from one single initial cell, the total number of cells observed was $N = 2^t$, where t is the number of cell cycles necessary to produce N cells from one single initial. Hence,

$$t = \log N / \log 2 \qquad (1)$$

(iv) the number of reverted cells (green), Ng, was counted and the following equation solved for p :

$$(1 - p)^t = (N - Ng)/ N \qquad (2)$$

Individuals to be irradiated were transplanted as cuttings from culture tubes into pots and exposed to the ^{60}Co source at a young vegetative stage (10 - 15 cm). The effects were observed in terms of number of reverted cells on leaves that had undergone divisions after the treatment.

II.3.1. Spontaneous Reversion Rate

The total number of cells observed was N = 19 311 280, of which Ng = 4462 were found to be reverted. Hence, from equations (1) and (2) ,

$$t = 24$$
$$p = 9.6 \times 10^{-6} \simeq 10^{-5} .$$

II.3.2. Dose-Response Relationship

The data made clear that the response was of two different types. However, the analysis of variance performed on the whole set of data only showed a highly significant linear component. This was too rough an analysis, because the weight of the linearity in the higher-dose range hindered the apparently non-linear response in the low-dose range. Therefore, the experiment was split into two parts : a low-dose (0 - 64 R) and a higher-dose (64 - 256 R) analysis, in which the response was found to be quadratic and linear, respectively. The response equations per cell were :

$$Y_{g1} = (2.86 + 0.675\ X + 0.288\ X^2)\ 10^{-4} \qquad (3)$$

$$Y_{g2} = (28.15 + 35.50\ X)\ 10^{-4} \qquad (4)$$

which can be considered as the proportions of reverted cells, or frequencies of reversion, in the low-dose and higher-dose ranges, respectively. In equation (3), X is coded 0, 1, 2, 4, 8 for 0, 8, 16, 32, 64 R, while in equation (4) X is coded 0, 1, 3 for 64, 128, 256 R.

A regression analysis performed in the low-dose range on the log-transformed data, from which the average value of the control had been subtracted before transformation, yielded a slope equal to 1.5 that was found to be significantly different from 1. This constitutes further evidence of the non-linearity of the response in this dose range.

The spontaneous reversion rate was estimated from equations(1), (2) and (3) , i.e.,

$$(1 - p)^{24} = 1 - 2.86 \times 10^{-4}$$

which yields $p = 1.2 \times 10^{-5} \simeq 10^{-5}$ as estimated previously.

It is worth noticing that Y_{g1} and Y_{g2} almost perfectly fit to one another, as it is shown below :

$$Y_{g1}\Big]_{X=8} = 2.7 \times 10^{-3} \quad ; \quad Y_{g2}\Big]_{X=0} = 2.8 \times 10^{-3}$$

In the low-dose range, an analysis of variance was also made on the data from irradiated material only, thus disregarding the control. The response remained quadratic, namely

$$Y'_{g1} = (4.60 + 0.578\ X + 0.369\ X^2)\ 10^{-4}$$

where X is coded 0, 1, 3, 7 for 8, 16, 32, 64 R, respectively. The extrapolated spontaneous reversion frequency is therefore

$$Y'_{g1}\Big]_{X=-1} = 4.39 \times 10^{-4}$$

which, from equations (1) and (2), yields the reversion rate p :

$$(1 - p)^{24} = 1 - 4.39 \times 10^{-4}$$

$$p = 1.8 \times 10^{-5}$$

This extrapolated p value is rather conspicuously overestimated, since it turns out to be almost the double of the value calculated from the control. This indicates that further study is needed to determine the shape of the response curve between 0 and 8 R. It should be noted that, too, Y'_{g1} fits well to Y_{g2} :

$$\left[Y'_{gl}\right]_{X=7} = 2.7 \times 10^{-3}$$

The instantaneous rate of increase of the response (efficiency) in the low-dose range is, from equation (3),

$$\frac{dY_{gl}}{dX} = (0.675 + 0.576\ X)\ 10^{-4}$$

where X is expressed in coded units. If X is to be expressed in roentgens, the equation above becomes :

$$r = (0.084 + 0.009\ X)\ 10^{-4} \qquad (5)$$

If the splitting of the experiment was correct, then the instantaneous rate of increase of the response at 64 R in the low-dose range should be approximately equal to the slope of the linear response found in the higher-dose range. From equations (5) and (4), these values are 0.66×10^{-4} and 0.55×10^{-4}, respectively. Since these figures are close enough to one another, it seems legitimate to think that the change of response from curvilinearity to linearity occurs around 64 R which, therefore, can be regarded as the smallest dose that yields maximum efficiency. This maximum efficiency remains constant up to 256 R. Further study is needed to investigate the shape of the response curve beyond 256 R.

From equation (5), the efficiency at 8 and 64 R is 0.16×10^{-4} and 0.66×10^{-4}, respectively. This means that, within this range, the efficiency quadruples when the dose is multiplied by eight.

Finally, the doubling dose for gamma-rays in this system was estimated from equation (3) to be 1.12 R.

All these results seem to be in good agreement with the findings of Sparrow et al. (1972, Science 176 : 916-918) on the effects of X-rays and Neutrons on Tradescantia stamen hair cells. Their results uphold the statement made earlier as to the necessity of further investigation of the response between 0 and 8 R.

Contractor: Carlsberg Laboratory, Department of Physiology
Contract No.: 124-74-1 BIODK
Head of research team: Prof. Dr. Diter von Wettstein
General subject of contract: Mutation Spectra of Eceriferum Genes

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An evaluation of the genetic health hazards of mutagens and their usefulness in plant and animal breeding requires precise information on the genetic variation induced by individual mutagens. We have therefore isolated over the last 15 years 1180 eceriferum mutants in barley, i.e. mutants with an organ specific change of the wax coating and assigned 992 recessive mutants to 59 gene loci spread across the seven barley chromosomes. The mutagens studied include acute and chronic irradiation with X- and γ-rays, neutrons, ethyleneimine and sulfonates. Highly significant differences in the mutation spectra are obtained. Whereas X-rays produce a rather unspecific mutation pattern, mutagens like neutrons, ethyleneimine and sulfonates hit certain genes more than others. The genes that mutate preferentially with the latter three mutagens are quite different ones.

With the aid of translocations, two point tests and three point tests 27 of the 59 genes have so far been assigned to chromosome arms. The location of genes that show specific affinity to certain mutagens is fundamental to further inquiries into the nature of mutagen specificities.

The mutants analysed genetically in this project serve as tools in studies on the structural organization, composition and biosynthesis of very long chain lipid molecules (fatty acids, hydrocarbons, diketones, alcohols, esters) in the epicuticular wax which constitutes the border of the plant surface and the surrounding atmosphere.

Results of Project No.: 1

Head of project and scientific staff: Prof. Dr. D. von Wettstein
Ms. U. Lundqvist
Dr. B. Søgaard
Dr. P. von Wettstein
Dr. A.G. Netting

Title of project: Mutation spectra of eceriferum genes after treatment with ionizing radiations and chemical mutagens

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The distribution of the 992 eceriferum mutants among the 59 loci is presented in the following table, in which the loci are arranged according to the number of alleles known:

Locus	c	q	u	i	za	a	zj	j	n	t	ze	b	e
Alleles	144	114	114	57	50	40	37	36	36	36	35	31	31
Locus	g	p	x	w	s	zc	zh	zi	d	zk	h	r	z
Alleles	27	23	19	16	13	11	10	10	9	8	5	5	5
Locus	zn	zo	zd	zp	yb	ye	f	v	zb	zs	zv	zl	yh
Alleles	5	5	4	4	4	4	3	3	3	3	3	2	2
Locus	zr	zt	zu	zz	yf	k	l	m	o	y	zf	zg	zw
Alleles	2	2	2	2	2	1	1	1	1	1	1	1	1
Locus	zx	zy	ya	yc	yd	yg	zq				Total		59
Alleles	1	1	1	1	1	1	1				Total		992

Locus cer-c, cer-q and cer-u are located in chromosome 4. Instances of simultaneous mutation of two or all three of these genes by neutrons suggest these loci to be closely linked. Chromosome 4 also carries cer-zh, cer-j and cer-zg. In chromosome 1 are found cer-a and cer-f, in chromosome 2 cer-v, cer-s, cer-g and cer-n, in chromosome 3 cer-r, cer-zd and cer-zn, in chromosome 5 cer-e and cer-zi, in chromosome 7 cer-b, cer-x, cer-zj, cer-i, cer-t, cer-zp and cer-w.

Four mutation spectra presented in Fig. 1 permit the following conclusions:

Loci cer-c, -q and -u mutate preferentially after treatment with the two alkylating chemical mutagens.

Locus cer-i is highly susceptible to neutron irradiation, but resistant towards the two chemical mutagens. Locus cer-j is resistant towards radiation, but easily mutated with the two chemical mutagens.

Locus cer-zj, cer-n and cer-t show hot spots with neutron irradiation, whereas cer-b and cer-g mutate preferentially after treatment with X-rays.

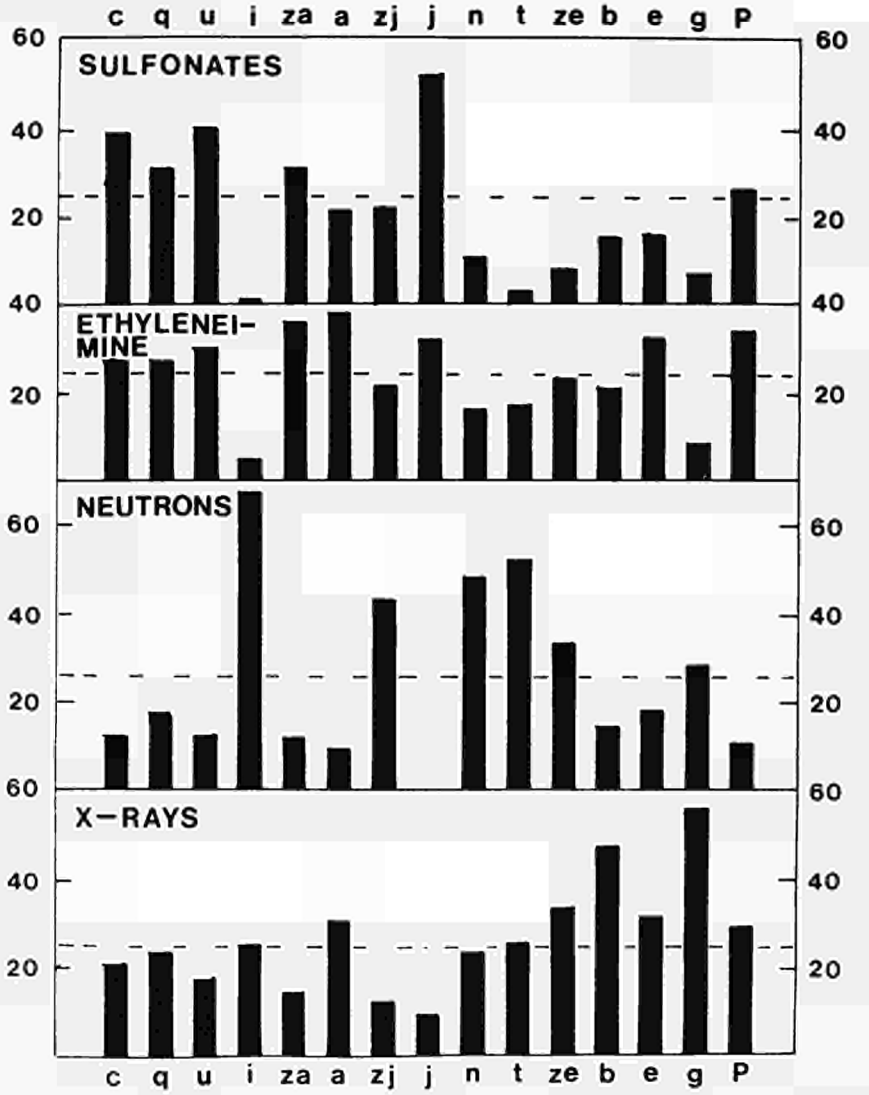


Fig. 1. Mutation spectra for eceriferum mutants induced with two physical and two chemical mutagens. For each of the fifteen loci with the highest number of mutations, the percentage of mutants induced with the four mutagens is given. The data were first normalized, so that an equal number of mutations is considered for each mutagen.

Publications

U. Lundqvist and D. von Wettstein. Stock list for the eceriferum mutants III. Barley Genetics Newsletter 5 (in press).

B.N. Giese. Effects of light and temperature on the composition of epicuticular wax of barley leaves. Phytochemistry (in press).

P. von Wettstein-Knowles. Gene mutation in barley inhibiting the production and use of C_{26} chains in epicuticular wax formation. FEBS Letters 42, 187-191 (1974).

B. Søgaard. Three-point tests on chromosomes 1 and 7. Barley Genetics Newsletter 4, 70-73 (1974).

B. Søgaard. The localization of eceriferum loci in barley III. Three-point tests of genes on chromosome 1 in barley. Hereditas 76, 41-48 (1974).

P. von Wettstein-Knowles. Ultrastructure and origin of epicuticular wax tubes. J. Ultrastructure Res. 46, 483-498 (1974).

Contractor: The Finsen Institute, Copenhagen.

Contract No.: 120-73-1-BIO-DK.

Head of research team: Mogens Faber.

General subject of Contract: Radiation Sensitivity of the Human Ovary.

During the year 1974 the work on the Radiation Sensitivity of the Human Ovary concerned itself with the following questions:

1. The normal development of the human ovary
2. Follicular atresia in the human ovary
3. Geography of follicle growth and atresia.

Results of Project:

Head of Project and scientific staff: Mogens Faber
Anne Grete Byskov
Hannah Peters
Ruth Himelstein-Braw.

Title of Project: Radiation Sensitivity of the Human Ovary.

1. In continuation of anatomical studies the morphology and growth pattern of human ovaries was described in ovaries of normal children, i.e. those that had died in accidents or after a short acute disease. It could be shown that growth and atresia goes on during all ages of childhood and that a quiescent ovary does not exist in normal children. This will be used as a basis for evaluating the effect of irradiation during childhood.

2. In order to be able to judge abnormal degeneration of follicles, the pattern of follicle atresia in human ovaries at different ages during childhood are being studied. It can be shown that atresia of follicles in normal ovaries occurs at all stages of follicle development. Few follicles become atretic in the beginning stages of follicle development while an increasing number becomes atretic as follicle growth progresses. This fact will be taken into consideration when ovaries from children that have received radiation will be evaluated.

3. In order to determine whether after irradiation abnormal areas of growth and degeneration are found in the ovary, it was necessary to investigate the spacial distribution of growing and degenerating follicles in normal ovaries. This is now in progress. A normal human ovary has been serially sectioned and the position of each growing or atretic follicle has been assigned a position on a coordinate system for computer analysis.

A program has been written to analyse accurately relationships of areas where growth and atresia occurs. A similar investigation will have to be done on an irradiated ovary.

Publications:

1. Lintern-Moore, S., H.Peters, G.P.M.Moore & M.Faber: Follicular development in the infant human ovary. J. Reprod. Fert. 1974, 39, 53-64.

2. Byskov, A.G.S.: Cell kinetic studies of follicular atresia in the mouse ovary. J. Reprod. Fert. 1974, 37, 277-285.

KURZZEITWIRKUNGEN (AKUTES STRAHLENSYNDROM UND SEINE BEHANDLUNG)

SHORT-TERM EFFECTS (ACUTE IRRADIATION SYNDROME AND ITS TREATMENT)

EFFETS A COURT TERME (SYNDROME AIGU D'IRRADIATION ET DE SON TRAITEMENT)

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

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

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

099-BIAB ULB Bruxelles (Brachet)

095-BIOB CEN Mol (Maisin)

Vertragspartner der Kommission:

Assoziationsvertrag

zwischen

der Europäischen Atomgemeinschaft

und

der Association Claude-Bernard
dem Istituto di Ricerche Farmacologiche "Mario Negri"
dem Land Baden-Württemberg
der Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek voor Gezondheid (TNO) und
der l'Université Libre de Bruxelles

Nr. des Vertrages: 088 - 72 - 1 BIA C

Leiter der Forschungsgruppen:

- Claude Bernard Association, Institut de Cancérologie et Immunogénétique, Villejuif: Prof. Dr. G. Mathé
- Istituto di Ricerche Farmacologiche "Mario Negri", Milano: Prof. Dr. S. Garattini
- Universität Ulm, Abteilung für Klinische Physiologie, Ulm: Prof. Dr. T. M. Fliedner
- Radiobiological Institute TNO, Rijswijk: Prof. Dr. D. W. van Bekkum
- Institut Jules Bordet, Bruxelles: Prof. Dr. H. Tagnon

Allgemeines Thema des Vertrages:

Consequences of Radiation Exposure : Prevention and Treatment of Pathological Effects

Allgemeine Darstellung der durchgeführten Arbeiten:

In 1974, the work of the laboratories participating in this association contract No. 088 - 72 - 1 BIA C was a continuation of the previous activities. They have been described in the Euratom Reports 1971 (EUR 4830 d - f - i - n - e), 1972 (EUR 4864 d - e - f - i - n) and 1973 (EUR 5138 d - e - f - i - n).

The collaborative research work of this Association Contract in 1974 was concerned, as before, with the following topics:

1. Evaluation of damage (mainly by means of hematological indicators) to the mammalian organism including man after external and / or internal exposure to ionizing radiation.

2. Treatment of hematopoietic failure - as seen after single high level or repeated or continuous low level whole body irradiation - by means of replacement (granulocyte transfusion, platelet transfusion) or substitution (bone marrow, stem-cell transfusion).
3. Cell system research relevant to the development of new diagnostic and therapeutic tools to evaluate or modify the consequences of ionizing radiation.

The three major topics of this Contract have been studied through close coordination and cooperation of the 5 participating research institutes in Villejuif, Milan, Ulm, Rijswijk and Brussels. The intensity of cooperation varied from project to project. However, the combined efforts represented the major European thrust in trying to improve existing or develop new tools to evaluate the damage observed after ionizing irradiation in human beings and to modify therapeutically bone marrow failure seen after low-level long-term or high-level short-term radiation exposure in man. Progress in these fields depends largely on the advancement and extension of knowledge in more basic research areas. Therefore, the diagnostic and therapeutic studies were supplemented by an extensive experimental programme of research in various animal models known to be suitable for the study of the particular, clinically relevant problem.

Research on the evaluation of damage, as seen after whole body exposure to ionizing radiation, using hematological approaches has been carried out by the Ulm Group. The concern for the evaluation of damage as seen after the incorporation of radioactive compounds, such as tritium, led to a continuation of the study on tritium toxicity . Tritium may well become, even moreso than at present, a serious problem in nuclear energy establishments. However, little is known about the biological effectiveness of various tritiated compounds from tritiated water to tritiated thymidine

or other DNA precursors. It became of considerable interest in 1974 to study the relationship of tritium incorporation to the stage of prenatal development of the organism using various sensitive parameters (eg. oocyte number) as end-points. "In vivo" and "in vitro" studies indicated the presence of both pluripotent and committed hemopoietic stem-cells in the peripheral blood. Since they are very sensitive to ionizing radiation with a D_o of about 105 rad, an attempt is being made to develop this characteristic into a new tool for estimating low level radiation exposure with a parameter that should have predictive value for remaining bone marrow function.

The improvement of existing and the development of new tools in the treatment of hemopoietic failure, as seen after whole body irradiation, was the goal of extensive investigations at the clinical level as well as at the animal experimental level.

The Group in Villejuif continued its extensive clinical programme directed towards the investigation of ways and means to improve the results of allogeneic bone marrow transplantation in man; this is still impaired by graft-versus-host disease. New approaches of this Group to prevent or treat secondary disease (at the pre-clinical experimental level) included anti-thymocyte serum, thymic chalone (to suppress lymphocyte proliferation), anti-recognition-site serum as well as enhancing serum.

The Group in Rijswijk continued its studies on the prevention of graft-versus-host disease using non-human primate and dog models. To this end, the studies of this group were concerned with the improvement of methods of selecting the most compatible host / donor combinations among unrelated rhesus monkeys. New approaches included the use of lymphocyte-defined (LD)

determinants; while the collection of appropriate LD-typing cells was possible, serological typing for LD-determinants was only in the beginning stages. This group also continued its work on an attempt to purify stem-cell-concentrates (for transplantation purposes) derived from random allogeneic donor dogs and considered - in a mouse model - the possibilities afforded by the use of fetal liver stem-cells to restore hemopoiesis. The Group in Ulm saw it to be its major task to further improve the possibilities of achieving and maintaining a gnotobiotic state in man by decontaminating the patients (for their conventional microflora) using antibiotics. This procedure now becomes of even greater importance by the finding that mice so rendered sterile by antibiotics show a markedly reduced mortality after allogeneic bone-marrow transplantation. This group continued the development of its preclinical dog model for an "allogeneic blood stem-cell bank". Since pluripotent hemopoietic stem-cells can be collected from the circulation and can be stored and preserved for long periods in liquid nitrogen (cryopreservation method) and since their transfusion into lethally irradiated allogeneic recipients can result in long-term chimaeras, this approach appears suitable for further development and the eventual transposition to the clinical level. In this context, the histocompatibility testing not only for DLA antigens but also for MLC loci has become of major importance. The Group in Milan contributed to the entire activity mainly through its competence in pharmacological research. It is clear that radiomimetic and immunosuppressive drugs are of major interest to the other participating laboratories dealing with the treatment of hemopoietic failure by allogeneic stem-cell transfusion. Therefore, this group conducted research devoted 1 to gaining a better understanding of the activity of radiomimetic immunodepressants in order to enable better and safer therapeutical use and 2 to investigating the mechanisms of action and best conditions of exploitation of agents which can increase the activity of the immune system.

All 5 research groups contributed significantly to the third topic of this association contract which is concerned with special cell system research. This research can be expected to provide the necessary physiological and pathophysiological background for advancing the investigations concerned with the attempt to improve diagnostic and therapeutic possibilities after radiation exposure in man.

The Group in Villejuif is concerned with questions relating to the concept of cell system regulation by inhibiting factors, termed chalones, and uses such factors to study the effect on immune-cell-systems.

The Group in Rijswijk carries out basic immunological research in the area of histocompatibility testing, taking into account 3 classes of antigens: conventional SD antigens, LD or MLR antigens and the still rather enigmatic la antigens. In addition, the work continued successfully on the identification and characterization of hemopoietic stem-cells by means of electronmicroscopy and the agar-culture technique.

The Group in Ulm is concerned mainly with the further characterization of the functional structure and characteristics of pluripotent hemopoietic stem-cells and with the question of feed-back regulation of the uncommitted and the various committed stem-cell pools. In this area, the toxic effects of radionuclides were compared to cycle specific cytotoxic agents. But, in addition, the studies using germfree, irradiated, marrow-transplanted and hypertransfused mice were continued and yielded valuable information on the factors affecting stem-cell differentiation at the level of uncommitted cells. In collaboration with the Group in Pesaro, directed by Prof. Lucarelli, certain aspects of the fetal stem-cell pools in the rat were investigated using the in vivo

culture system provided by the diffusion chambers.

The Group in Brussels contributed to the whole effort by the continuation of studies on the kinetics and regulation of cell proliferation of both normal and pathological bone marrow cells. Of great interest in this programme are the mechanisms that trigger latent resting cells to perform "unscheduled" DNA synthesis. Furthermore, studies relating to the elucidation of mechanisms that help to release cells from the marrow into the blood and that control the influx of blood (-stem) -cells back into the marrow are of particular interest.

The cooperation and coordination of efforts in this Association is executed by different mechanisms. There are bilateral and multilateral research projects in which the institutions of this contract as well as other laboratories participate. For instance, the gnotobiotic research work is coordinated by the EORTC-Gnotobiotic Project Group (President: Dr. Dietrich, Ulm). The work on the characterization of hemopoietic stem-cells is discussed and synchronized by means of the EORTC-Stem-Cell-Club (Convener: Dr. van Putten, Rijswijk). The pre-clinical bone marrow transplantation programme is coordinated through a "dog bone marrow transplantation study group". A task force on cryopreservation of platelets met in 1974 and will continue its coordinating work. The members of the participating laboratories met in 1974 at several workshops to exchange their views and maintain collaborative links.

Thus, there is no doubt that the efforts made in the participating laboratories of this contract represent a significant concerted action and a major thrust in the field of the investigation of "short-term effects" of ionizing radiation.

Contractant de la Commission: Association Claude-Bernard, Institut de Cancérologie et d'Immunogénétique

N° du contrat: 088.72.I.BIAC

Chef du Groupe de Recherche: Professeur Georges MATHE, Directeur de l'Institut de Cancérologie et d'Immunogénétique

Thème général du contrat: Prévention et traitement des états pathologiques secondaires à l'irradiation.

Nous avons, dans l'année écoulée, poursuivi les travaux sur la prévention et le traitement de la maladie secondaire déclenchée après une greffe de moelle osseuse allogénique par la réaction du greffon contre l'hôte.

Le modèle expérimental utilisé est celui de souris F1 (DBA/2 C57Bl/6) irradiées à la dose de 950 rads et recevant $I0^7$ cellules de moelle osseuse et 0,1 ml de sang total provenant de donneurs C57Bl/6. Ce modèle a été choisi pour se rapprocher le plus possible des conditions pratiques de la greffe de moelle allogénique chez l'homme.

Nous avons mené nos recherches dans 3 directions essentielles: le traitement des cellules C57Bl/6 in vitro, avant la greffe, le traitement précoce des receveurs après la greffe, le traitement tardif des receveurs après la greffe, ceci, également, afin de se rapprocher le plus possible des traitements applicables en clinique humaine où la manipulation de donneurs normaux est évidemment impossible.

Nous avons utilisé 3 types de traitements non spécifiques un sérum de lapin anti thymocytes de souris, les fragments FAB obtenus à partir de ce même sérum, la chalone thymique, isolée, étudiée et purifiée dans notre laboratoire. Nous avons utilisé 3 types de traitements spécifiques: un sérum anti-cellules du receveur, dont nous espérions une action facilitante, un sérum anti récepteur spécifique des lymphocytes T du donneur, et des antigènes H-2 solubles. Le sérum anti cellules du receveur est obtenu par l'immunisation de souris C57Bl/6 avec des cellules lyophilisées de rates de souris DBA/2. Le sérum anti récepteur spécifique est produit en injectant à des F1 (C57Bl/6 DBA/2) non irradiées $5.I0^6$ cellules de rates C57Bl/6, les cellules C57Bl/6 ont un récepteur spécifique pour les antigènes DBA/2 que n'ont pas les cellules F1, le receveur F1 doit donc s'immuniser contre ces récepteurs spécifiques des membranes des lymphocytes T C57Bl/6. Les antigènes H-2 solubles sont obtenus à partir de foie de souris Balb/c selon des méthodes déjà décrites dans notre laboratoire, donc à partir de cellules allogéniques par rapport aux F1 (C57Bl/6xDBA/2) mais ayant le même génotype H-2 que les souris DBA/2.

Résultats du projet N°088.72.1.BIAC

Chef du projet : Professeur Georges MATHE

Collaborateurs scientifiques: Léon SCHWARZENBERG - Marcel HAYAT - Pierre POUILLART -

Titre du projet: Prévention et traitement des états pathologiques secondaires à l'irradiation.

Le sérum hétéro spécifique de lapin anti thymocytes de souris ne s'est montré efficace ni in vitro ni in vivo, Ces résultats peuvent paraitre surprenants si l'on se rappelle la disparition totale de la maladie secondaire provoquée par le conditionnement du receveur par le seul sérum anti lymphocytes, mais ils montrent que cette disparition de la maladie secondaire, chez la souris et chez l'Homme, lorsoque le receveur est conditionné par le sérum anti lymphocytes seul est bien dûe au caractère dissocié de la greffe allogénique réussie dans ces conditions, donc à une action plus sélective et moins profonde que celle de l'irradiation totale ou des anti mitotiques à fortes doses sur le système lymphoïde du receveur. Quoique négatifs, ces résultats nous paraissent importants, car ils montrent que l'utilité du sérum anti lymphocytaire se situe essentiellement avant la greffe et sur le receveur, et qu'il est donc inutile de prolonger son administration après la greffe pour agir sur le greffon.

Les mêmes résultats négatifs sont obtenus par les fragments FAB obtenus à partir du sérum anti lymphocytes.

L'utilisation de sérum anti récepteur spécifique est évidemment une idée séduisante puisqu'un tel immun sérum pourrait faire le tri dans un greffon hématopoïétique allogénique entre les granulocytes qui ne possèdent pas de tels récepteurs et les lymphocytes qui les possèdent. Les premiers résultats que nous rapportons sont malheureusement complètement négatifs.

L'utilisation d'antigènes d'histocompatibilité solubles a par contre, donné des résultats très positifs. Les donneurs des cellules utilisées pour obtenir ces antigènes différent des receveurs et des donneurs de cellules destinées à être greffées, mais possèdent le même génome d'histocompatibilité majeure que le receveur. Il y a donc là un système très souple, utilisable chez l'Homme dans des conditions

privilégiées, et cette méthode peut, comme nous l'avons montré, en collaboration avec Brent, additionner ses effets à ceux d'autres traitements, non spécifiques, de la maladie secondaire. Il est à noter que si les antigènes solubles sont obtenus à partir des cellules mêmes du donneur, aucun effet n'est enregistré ce qui montre bien qu'il s'agit là d'un traitement spécifique de la maladie secondaire.

Nous avons obtenu également des résultats positifs avec la chalone thymique dans la prévention de la maladie secondaire, mais non dans son traitement. Les animaux sont protégés si le produit est mis in vitro, au contact des cellules avant leur greffe ou s'il est administré jusqu'au jour I0. La chalone thymique donnée du jour 20 au jour 30 n'a pas d'effet décelable, contrairement aux antigènes solubles qui sont efficaces si leur administration est retardée et le sont nettement moins si leur administration est précoce. Le traitement par une chalone est évidemment un traitement très prometteur en clinique: les chalones ont, en effet, une spécificité de tissu et non d'espèce, et le produit actif chez la souris pourrait l'être chez l'Homme.

Nos premiers résultats semblent montrer que les antigènes solubles et la chalone thymique agissent selon des mécanismes différents et d'ailleurs à des moments différents de la chaine des évènements cellulaires qui aboutissent à la réaction du greffon contre l'hôte et à la maladie secondaire. Les antigènes d'histocompatibilité solubles sont surtout efficaces dans l'incubation "in vitro", peut-être en favorisant les clones cellulaires responsables de réactions non lytiques et peut-être même protectrices, vis à vis des cellules du futur receveur. Dans les premiers jours de la greffe, la chalone thymique donne ses résultats les plus nets comme il est assez logique puisque c'est dans ces premiers jours que s'établit la prolifération de cellules T du greffon, responsables de réactions importantes pour le déclenchement de la maladie secondaire. Une fois établie, cette réaction du greffon contre l'hôte ne semble plus pouvoir être contrôlée suffisamment par la chalone thymique, mais l'administration d'antigènes solubles semble encore capable de dévier cette réaction du greffon contre l'hôte vers un type de réaction non létal pour le receveur.

Nous avons également obtenu des résultats positifs mais faiblement positifs, avec un immun sérum anti receveurs qui a donc un certain pouvoir de "facilitation" vis à vis du receveur.

EURATOM - CONTRAT N°088.72.I.BIAC. G. MATHE - I.C.I.G.

PUBLICATIONS I974

MATHE G., SCHWARZENBERG L., KIGER N., FLORENTIN I., HALLE-PANNENKO O et GARCIA-GIRALT E. Bone marrow transplantation for aplasias and leukemias. p. 33 in "Clinical immunobiology (F.H. Bach et R.A Good, eds). vol. 2, New-York, I974, Academic Press.

MATHE G. et SCHWARZENBERG L. Bone marrow transplantation in France I958-I973. Transplant. Proc., I974, 6, 335.

MATHE G., HALLE-PANNENKO O., KIGER N., FLORENTIN I et BOURUT C. Comparison of the effects of three non specific means: ATS, ATS-Fab fragments and thymic chalone on the prevention and treatment of bone marrow graft secondary disease in the clinical mouse model. Biomedicine, I974 (sous presse).

HALLE-PANNENKO O., MATHE G., GOUJET-ZALC C., MARTYRE M.C. et BOURUT C. Comparison of the effect of specific means: anti recognition site serum, enhancing serum, and soluble H-2 antigen for the prevention and treatment of bone marrow graft secondary disease in a preclinical mouse model. Exp. Hematol., I974, sous presse.

Istituto di Ricerche Farmacologiche "Mario Negri"

Contract Number : 088-72-1- BIAC

Head : Silvio Garattini, M.D.

CONSEQUENCES OF RADIATION EXPOSURE, PREVENTION AND TREATMENT OF PATHOLOGICAL EFFECTS.

The studies conducted during this year have followed two main lines,the first devoted to gain a better knowledge of the activity of radiomimetic immunodepressant with the aim of ultimately reaching a better and safer therapeutical use of these agents , whereas the second line has concentrated on investigating the mechanisms of action and best conditions of exploitation of agents which can increase the activity of the immune system. These lines of research appear interconnected and appear both of importance in the atteppt for always more effective treatment of immediate or delayed radiation exposure consequences . In this frame, a further phase in our studies of drug interaction in cytotoxic treatment has been completed with the revelation of complex interactions between methotrexate and steroids in vivo . In the course of studies on the characterization of the Adriamycin and Daunomycin, cytotoxic agents of current wide clinical interest , insights into the in vivo role of an important immune effector mechanism, the so-called phenomenon of antibody-dependent cellular cytotoxicity, have been obtained ,thus contributing to a better clarification of the multifaceted biology of the immune system response to foreign tissue . Since agents exist

which dispaly an operational selectivity for the various immune effector mechanisms , it is hoped that these findings may ultimately be helpful in devising more effective means to use immunodepressants as well as to understand in more detail the biological basis of the immunodepression induced by radiomimetic agents.

Along the second principal line of research, the characterization of a novel immunomodulator with interesting potential for clinical use has been obtained . Of special note in the frame of the overall aim of this multigroup project appears, for instance, the possibility of reducing the recovery period of immune negativity induced by cytotoxic , i.e. of positively influencing one of the major consequences of exposure to radiation or radiation-like agents .With the same hope,novel evidence has been obtained on the mechanisms of action of another immunostimulator of frequent clinical use ;again, the type of knowledge acquired through this type of studies may be instrumental towards a more effective treatments of radiation exposure . Lastly , the influence of a technical variable , possessing also potential biological significance , in the performance of a standard in vitro test employed to obtain information on the host immune status, has been revealed .

Project N. RESULTS OF PROJECT N. 1

Title : DRUG INTERACTION IN CYTOTOXIC TREATMENT : II .

Scientific Staff :
F.Spreafico, M.D., A.Mantovani, M.D., A.Vecchi, Ph.D.

This type of studies is the continuation of a line of investigations already pursued in the past by this laboratory and which stems from our desire to always better rationalize immuno-suppressive treatments,when it involves multiple drug combinations . The possible interactions between methotrexate (MTX), a widely employed cytotoxic agent , and other drugs with which it can frequently be associated in the clinic, such as steroids and antibiotics,were examined. In vitro studies have in fact shown that the active intracellular transport of MTX by leukemia cells can be markedly interferred with by Prednisone , Hydroxycorticosterone,Penicillin G , Kanamycin and Cephalothin . The effects of such an interaction on the in vivo immunosuppressive and antileukemic activities of MTX were thus assessed . It has been found that no significant changes in both these cytotoxic activities of MTX were induced by association, in various schedules of treatment, with clinical doses of the above mentioned antibiotics , leading to the conclusion that the in vitro interaction observed is of probable no significance in clinical treatments . On the other hand, significant modifications in MTX activity were seen by its association with both steroids . In fact, the antileukemic effect of MTX can be markedly reduced by pre- and posttreatments with hydrocortisone and a similar effect is found with pretreatments of prednisone . As regards the immunosuppressive activity of MTX , the interaction can lead to a reduction or to an enhancement of immuno-depression by the antifolate depending on the relative timing of steroid and MTX injection . The results obtained point towards the conclusion that the immunodepression induced by the steroids is responsible for the reduction in MTX antileukemic activity,since the latter drug requires a partecipation of

host' immunity to display its full effect . As regards the changes in immuno-depression ,a combination of two mechanisms, i.e. interference in MTX uptake and summation of subthreshold activities , seem the more likely explanation of the changing direction of the interaction between MTX and steroids depending on this relative timing of administration . In any case ,the possibility that this combination may be followed by unexpected, qualitative and quantitative results , suggest caution in its therapeutical use .

Project N. RESULTS OF PROJECT N. 2

Title ON THE IN VIVO ROLE OF ANTIBODY-DEPENDENT CELLULAR CYTOTOXICITY

Name of Investigatores : F.Spreafico, M.D., A.Mantovani, M.D., A.Vecchi,Ph.D.

Antibody-dependent cellular cytotoxicity (ADCC) has recently been suggested as a possible additional effector mechanism in normal and neoplastic foreign tissue rejection processes; however ,only in vitro evidence in support of this contention was available . In order to more fully characterise its possible in vivo role and in the frame of a coordinated study on the mechanisms of action of Adriamycin (Adria) and Daunomycin (Dauno), evidence has been obtained that ADCC may indeed function as a crucial effector mechanism in allogeneic tissue rejection . It was observed that Adria-pretreated mice, survived significantly longer the inoculation of high dose of an allogeneic tumor than animals given an equitoxic dose of Dauno . An analysis of the activity of the specific immune effector mechanisms in these host revealed that Adria inhibited cell-mediated cytotoxicity more profoundly than Dauno, that the levels of complement dependent cytolytic antibodies were equally depressed by both drugs, whereas the capacity to mount ADCC was significantly more active in Adria- pretreated hosts than in Dauno- injected ones . Thus the only immune parameter positively correlated with the longer survival of Adria-pretreated animals was the activity of ADCC,whose time-course showed also a clear correlation with the higher cell killing in these mice . Similar results are also being obtained in a GVH system . These results and others obtained in concomitant studies contribute on the one hand to a clarification of the in vivo role of ADCC and at the same time, help in defining the mode of action of Adria and Dauno, cytotoxic compounds of great interest at the present time .

Project N. RESULTS OF PROJECT N. 3

Title CHARACTERIZATION OF THE IMMUNOMODULATOR, LEVAMISOLE

Name of Investigators : F.Spreafico, M.D., A.Mantovani,M.D.,A.Vecchi,Ph.D
A.Tagliabue,Ph.D.

Studies have been conducted to characterize in tumorous and non-tumorous model systems, Levamisole (Leva), a new atoxic immunomodulator of synthetic origin. This compound,in appropriate dosages, can increase either the peak response or prolong the primary and secondary immune responses to standard antigens in adult and especially in aged , immunologically depressed mice. In rats , the incidence, severity, and duration of autoimmune disease such as experimental encephalomyelitis, can be enhanced by administration of this drug, which can also sharply hasten the recovery from previous immunosuppression by cytotoxic agents . In tumorous oonditions, cures of leukemia-bearing mice can be obtained by associating this drug to irradiated tumor cells ;Leva can reduce metastasis formation in a spontaneously disseminating mouse tumour model, where a definite synergism with cures can be obtained by its combination with chemotherapy or previous surgery . Leva is not different from other immunomodulators in that, by appropriate treatments,tumor growth enhancement can also be observed,sustained by a depression of cell-mediated cytotoxicity and higher serum blocking activity . Immunoadjuvant activity of this agent is not accompanied by an increase in spleen cellularity nor by significant alterations in the numbers of the various lymphocyte subpopulations in this organ. An increase in cyclic-AMP but especially in c-GMP follows injections of this adjuvant and studies are in progress to correlate its activity and levels in plasma and target organs by a newly developed ,specific detection method which exploits gas chromatography-mass spectrometry . The latter type of investigations may help in elucidating the reasons why impredictable oscillations in activity can be observed employing Leva even in the same,well controlled experimental conditions . On the basis of these data,Levamisole appears a compound with interesting characteristics which in view of its activity and non toxicity,may find promising applications as an immunomodulator .

Project N. RESULTS OF PROJECT N. 4

Title THE MECHANISM OF ACTION OF CORYNEBACTERIUM PARVUM

Name of Investigators : A.Mantovani, M.D., A.Tagliabue, Ph.D.
F.Spreafico, M.D.

In our effort to investigate the interaction of immunostimulants with the lymphoid system,a study was carried out of the modifications in immune effector mechanisms accompanying increased antitumor resistance in mice after injections of C.Parvum. At variance with claims by others that intravenous C.Parvum does not lead to the development of systemic antineoplastic immunity and only nonspecific mechanisms are activated, we have observed that in C3H mice transplanted with the L 1210 leukemia, specific immune responses are more important than activated macrophages in mediating cell killing . In fact in these animals, a marked increase in cell mediated cytotoxicity and in the capacity of spleen cells to mediate antibody-dependent cellular cytotoxicity were observed without significant modifications in the circulating levels of arming,potentiating and direct cytolytic antibodies . These changes are accompanied by marked increase in spleen cellularity attributable to enhanced lymphocyte trapping , which carries as a consequence a greater influx of non-T cells and a decrease in T-cell percentage, which are very long lasting . The modifications observed in immune effector mechanisms can be explained by an activation of B elements induced by C.Parvum; such an activation together with that of macrophages and an inhibition of T cell reactivity, seem to represent the essential features of C.Parvum mode of action . In the course of this studies,evidence has also been obtained to the effect that the cells mediating antibody-dependent cellular cytotoxicity cannot be inhibited by blocking factors ; in other words,blocking factors act only on T-lymphocytes while non-T elements are insensitive to this inhibition . This novel finding is believed to offer a possibly significant contribution to a better understanding of the complex biology of the immune cells in their effector functions.

Project N. RESULTS OF PROJECT N. 5

Title THE INFLUENCE OF THE ATTACKER : TARGET CELL RATIO ON SERUM EFFECTS ON IN VITRO CELL-MEDIATED CYTOTOXICITY

Name of Investigators : F.Spreafico, M.D., A.Mantovani, M.D.

In vitro assays of cell-mediated cytotoxicity are important methods for the evaluation of the immune status of the host. It is known that serum can significantly modify the cytotoxic capacity of immune cells towards their targets either blocking or increasing this activity ;it has also been shown that the same serum can variably influence cell-mediated cytotoxicity (CMC) depending on the day of cell collection after antigen stimulation,which is attributable to the changing nature and/or proportion of T and B attacker cells in many experimental conditions . Our study illustrates the importance of an additional variable in affecting the final results observed in CMC assays in the presence of serum, namely the attacker : target cell (A:T) ratio . Employing in fact C3H spleen cells immune to L 1210 leukemia and a standard,short-term ^{51}Cr release test, it was observed that the same immune, arming serum could significantly potentiate CMC when low ($\leq$ 20:1) A:T ratios were used, whereas showing blocking if higher (100:1) A:T ratios were employed . These findings thus reveal that for a meaningful evaluation of in vivo conditions through in vitro tests, a number of technical variables must be taken into consideration and provide the basis for the interpretation of previously unexplained discrepancies between the in vivo and in vitro observations . Two mechanisms also proposed to explain the observed findings , based on the probable coexistence in the sera showing this activity of blocking factors and arming/ potentiating antibodies . At low A:T ratio a relative excess of antigenic sites exists , it is then conceivable that their masking by blocking factors would be insufficient to cause reduction in CMC whereas recruitment by potentiating antibodies of additional cytotoxic cells would lead to a greater cell killing as observed .Alternatively, if it is assumed that antigen or antigen-antibody complexes are blocking , the greater cell lysis obtained with

higher A:T ratios would lead to an higher local production of blocking factors with an inherent dampening effect on CMC , not observable at low A:T ratios, where a partial cancelling of T-cell cytotoxicity would still be supplemented by the recruitment by potentiating antibody of non-T effector cells .

EURATOM CONTRACT No. 088-72-1 BIA C

List of papers

ACTIVITY OF CYCLOPHOSPHAMIDE AND 1-METHYLNITROSOUREA ON EHRLICH CARCINOMA TRANSPLANTED IN DIFFERENT SITES. CORRELATION BETWEEN DRUG LEVEL AND TUMOR INHIBITION .
Bossi A., Colombo T., Donelli M.G., Garattini S.: Biochem.Pharmacol. accepted for publication . (1974)

INTERACTIONS OF ANTICANCER AGENTS WITH OTHER DRUGS .
Garattini S., Bartosek I., Donelli M.G., Spreafico F. Proceed. 27th Symp. on Fundamental Cancer Research - February 27-March 1, Houston, Texas, USA . (1974)

CYTOTOXIC EFFECT IN VITRO AND TUMOR VOLUME REDUCTION IN VIVO INDUCED BY CHEMOTHERAPEUTIC AGENTS .
Morasca L., Balconi G., Erba E., Lelieveld L., Van Putten L.M.
Europ. J. Cancer : accepted for publication . (1974) .

APPLICATION OF GAS-CHROMATOGRAPHY-CHEMICAL IONIZATION-MASS FRAGMENTOGRAPHY IN THE EVALUATION OF BASES AND NUCLEOSIDE ANALOGUES USED IN CANCER CHEMOTHERAPY .
Pantarotto C., Martini A., Belvedere G., Bossi A., Donelli M.G., Frigerio A. : J. Chrom. 99 : 519-527 (1974) .

Vertragspartner der Kommission:

Land Baden-Württemberg, vertreten durch die Universität Ulm

Nr. des Vertrages: 088 - 72 - 1 BIA D

Leiter der Forschungsgruppe:

Prof. Dr. Theodor M. Fliedner

Allgemeines Thema des Vertrages:

Effects of ionizing radiation on mammalian organisms and their treatment.

Allgemeine Darstellung der durchgeführten Arbeiten:

The work of the research group in Ulm was developed along the general lines presented in the working plan for 1974. It was the objective of the work in the framework of the EURATOM multinational association contract to improve existing and develop new tools to evaluate radiation damage and to treat its effects on hemopoiesis. The achievment of this goal was aided greatly by the establishment of the Sonderforschungsbereich "Zellsystemphysiologie" of the Deutsche Forschungsgemeinschaft, which supports basic research and thus provides the soil for the fruitful execution of the more applied EURATOM-Association programme.

The EURATOM-Association programme of the Ulm group contributed to the 3 main topics of the overall multinational Association.

In relation to point 1 (Evaluation of radiation damage), progress was made in the attempt to evaluate the apparent difference between randomly distributed and DNA-bound tritium. In addition, there is evidence that the culturing of hemopoietic blood stem cells by "in vitro" methods and in diffusion chambers can be utilized to detect low level radiation exposure.

With respect to point 2 (Treatment of radiation injury to hematopoiesis), the Ulm group contributed specifically to the testing of the hypothesis that the induction and main-

tenance of a gnotobiotic state is possible in man and reduces the incidence of infection in granulocytopenic states. Furthermore, a gnotobiotic state in mice was shown to suppress clinical evidence of graft-versus-host disease in allogeneic bone marrow chimaeras. The patient programme is carried out in the framework of the EORTC-Gnotobiotic-Project Group according to an accepted randomization protocol. The randomization for the entire group as well as the statistical evaluation of data is done by the Division of Medical Statistics of the University of Ulm and aided by the Computer of the University Data Center. A major programme in the context of "Therapy research" is the allogeneic hemopoietic blood stem-cell transplantation programme using the dog as a preclinical model. This model has been developed further and indicates strongly the feasibility of a "Cryopreservation Blood Stem Cell Bank" to be used for restoration of hemopoiesis after radiation - induced bone marrow failure. The data indicate that longterm chimaeras are possible and that it is now necessary to diminish or prevent graft-versus-host disease caused by the rather large lymphocytic contamination present when blood leukocytes are used as a stem-cell source. In this area, significant contributions were made in the field of histocompatibility testing in dogs.

Last, but not least, the Ulm group executed a number of basic studies in area 3 (Cell system research) to elucidate the interrelationship between committed and uncommitted hemopoietic stem-cells and their regulatory mechanism. These are important for the understanding of the pattern of marrow regeneration after stem-cell transfusion which may perhaps lead to an increased efficiency of blood cell regeneration. Several models in normal and germfree mice were chosen and the modern arsenal of stem-cell manipulation tests employed. In collaboration with the laboratory of Prof. Lucarelli in Pesaro, a research project on the characterization of fetal stem-cells (liver and marrow) was initiated and shows significant progress.

Ergebnisse des Projektes Nr. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter:

W. Schreml, T. M. Fliedner, W. Calvo with C. Bruch, R. J. Haas, E. B. Harriss and W. Nothdurft.

Titel des Projektes:

Evaluation of radiation damage after external and internal radiation exposure

Results:

This project, in 1974, continued 1 the study of the early and late tritium toxicity in rats and initiated 2 a programme on the use of blood stem-cell determinations to detect radiation damage.

From the data gathered on newborn rats continuously exposed to ^{3}H-TdR or HTO during pregnancy, estimations of the internal relative biological effectiveness have been made for various systems in the developing rat, as described in the 1973 report.

Two approaches were used during the past year in order to further evaluate the apparent difference between randomly distributed and DNA-bound tritium. Firstly, it appeared necessary to define the exact time at which specific radiobiological damage is induced in the developing rat and to compare the effective dose at this point. The experimental design involved short-term continuous infusion into pregnant rats for 2 days, either from day 11 to 13 or from day 13 to 15. ^{3}H-TdR and HTO were given in an amount which had been shown to reduce the oocyte number to approximately 10% when given from day 8 until term.

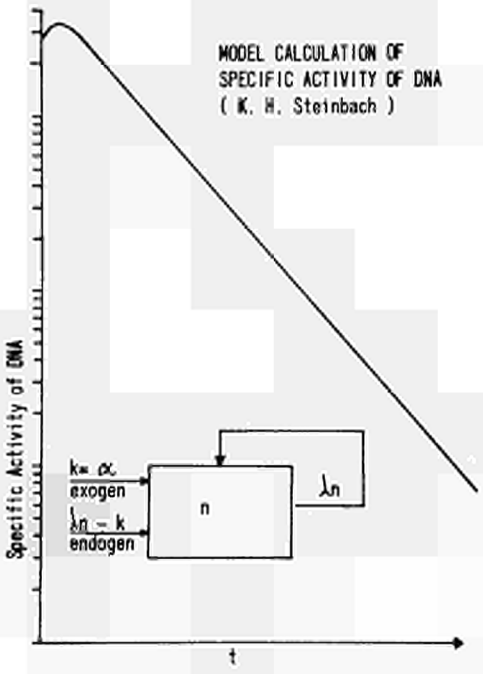
The oocyte number was then determined in the newborn animals. After HTO, a reduction of oocyte number to about 50% occurred in both experimental groups. Measurement of the

circulating tritium activity in these animals showed that similar levels of HTO were present around day 14 in both groups.

In contrast, animals receiving ^{3}H-TdR from day 11 to 13 showed little depression of oocyte number, while infusion from day 13 to 15 reduced the oocate number to less than 10% of normal. This fact indicates that the tritium dose to the oocytes has to be difined for the critical period of the oocyte system, i. e., for days 14/15 of pregnancy. Biochemical data are presently being studies after short-term infusion for evaluation of this point.

A combined experimental and mathematical approach was utilized in order to simulate the build-up of volatile and DNA-bound tritium activity during the continuous infusion period. This model takes into account measurements of T/2 for ^{3}H-TdR in pregnant rats and the distribution of ^{3}H-TdR in pregnant rats and the distribution of ^{3}H-TdR between mother and fetus in relation to the specific activity of the administered ^{3}H-TdR. These data are intended to give information on the pool size of DNA-precursors in the mother-fetus-system and on the ratio of "de novo" to salvage pathway for TdR in the developing rat. The mathematical model accounts for the increasing amount of thymidine necessary for DNA-duplication while the fetus is rapidly growing. In the most simple model, assuming a constant supply of exogenous TdR, this TdR represents a major portion of the necessary DNA precursors at early stages of development (while the fetus is very small); on the other hand, during the later part of the continuous infusion, the de novo synthesis has to provide the predominant proportion of the TTP. This leads to a high specific activity of DNA at early time-points which decreases during the course of continuous infusion. (Fig. 1)

Fig. 1



These studies show that the estimation of an effective tritium dose has to be considered during the time when the biological effect occurs.

In 1974, a programme was initiated at the pre-clinical level to use quantitative and qualitative properties of circulating pluripotent stem-cells in dogs to detect and evaluate radiation damage to the hemopoietic tissue. Since it appears likely that blood stem-cells reflect (in their concentration in the peripheral blood) the equilibrium and / or migration dynamics between all sites of active hemopoiesis in the skeleton, whole-body X-irradiation should have a significant effect on the concentration of blood stem-cells. As

a consequence of hemopoietic restoration in the bone marrow after radiation exposure, the blood stem-cell concentration should return to normal, although some qualitative aspects, such as the suicidal fraction, should remain abnormal, as long as the process of regeneration is not completed. The result of pilot studies - using the soft-agar - culture technique to demonstrate the presence of committed stem-cells (CFUc) support the hypothesis. After very high doses of radiation, the CFUc disappear completely from the blood in a very short time. They return in relation to the pattern of marrow regeneration, but it takes several weeks before the ^{3}H-TdR-suicidal fraction returns to normal. Thus, further work has to be done to find the threshold dose for an effect on the blood stem-cell pool and to characterize its stem-cell population qualitatively as a function of time after single or repeated radiation exposure.

Ergebnisse des Projektes Nr. 2:

Leiter des Projektes und wissenschaftliche Mitarbeiter:

T. M. Fliedner, H. D. Flad, W. Nothdurft with C. Bruch, W. Calvo, S. F. Goldmann, E. B. Harriss, E. Herbst, E. Hügl, R. P. Huget, M, Körbling, K. von Loringhoven, W. M. Ross and H. P. Schnappauf.

Titel des Projektes:

Blood stem-cell transfusion into lethally irradiated dogs as a preclinical model for the treatment of radiation-induced hemopoietic failure.

Results:

The objective of this project is to work out in a preclinical model - using the dog as an experimental animal - the prerequisites for the establishment of a "cryopreservation hemopoietic blood stem-cell bank" as a conditional necessity for the availability of an "unlimited" supply of hemopoietic stem-cells for the treatment of radiation-induced hemopoietic failure. Since blood stem-cells are present among mononuclear leukocytes, it is feasible to collect them together with the leukocytes from the peripheral blood either in a whole blood pack or by continuous leukocytapheresis. Autologous studies had provided evidence that DMSO cryopreserved blood stem-cells were almost as efficient as fresh mononuclear cells in restoring hemopoiesis after 1 200 R (midpoint dose) whole body X-irradiation and that the long-term survival of such transplanted animals was possible with consistency.

Therefore, in 1974, emphasis was placed on the question what prerequisites exist for a long-term allogeneic blood stem-cell graft. A pilot study had shown that it was possible to achieve a long-term allogeneic chimaera (sex-chromosome study) despite the relatively high proportion of lymphocytes to stem-cells in a leukocyte suspension. This dog showed signs of GvH-disease, but did not require continuing immunosuppressive therapy.

Thus, the main programme in 1974 dealt with the problem of allogeneic blood stem-cell transfusion under well-defined histocompatibility conditions. DL-A mismatched and DL-A matched donor-recipient combinations have been compared with respect to engraftment, clinical and / or pathological symptoms of graft-versus-host-reaction (GvHR) and survival. Furthermore, the major histocompatibility complex of the dog has been further defined using seriological typing, the mixed lymphocyte culture technique and cell-mediated lympholysis.

The results obtained may be summarized as follows:

1. Transfusion of allogeneic blood leukocytes (Ref.1):
   Blood leukocytes were collected from beagle dogs by means of an IBM Cell Separator, then frozen and stored at -196°C. They were thawed and various numbers of mononuclear cells were transfused into lethally irradiated (1200 R) recipient dogs. The results show that in 5 DL-A and MLC mismatched donor-recipient combinations all recipients died within 7 to 9 days after transplantation. (Table I)

   Table I :

   DL-A mismatched donor-recipient combinations

combination	cells transpl. /kg b.w. x 10^9	"take"	GvHR	survival (days)
1	1.7	+	+	8
2	0.5	(+)	+	7
3	0.7	+	+	8
4	0.7	+	+	8
5	0.6	+	+	9

   As a further step, DL-A identical, MLC negative donor-recipient combinations were selected. In these combinations 4 out of 10 dogs survived 20 days or longer. (Table II)

Table II

DL-A identical, MLC negative donor-recipient combinations

Recipient No.	mononucl. cells transplanted /kg b.w. x 10^9	"take"	GvHR	survival (days)
67039	0.5	+	+	14
92127	1.1	+	+	15
92129	1.1	+	beg.	8
97073	1.2	+	+	8
67038	1.3	+	beg.	6
127075	1.3	+	+	27
67029	1.5	+	mild, chron.	66
57017	1.5	+	mild, chron.	581 x
97072	1.7	+	ø	20
67030	2.8	+	+	17

x = per Jan. 31, 1975

Although the mean survival time (excluding recipient No. 57017) could be increased to 20.1 days (n = 9), 9 out of 10 dogs still developed clinical and / or histological symptoms of GvHR despite DL-A identity and MLC negativity. It can be concluded from these data that other genetic loci outside the major histocompatibility complex (MHC) for antigens in various tissues (skin, gut, liver and others) strongly contribute to the development of GvH -lesions.

2. Histocompatibility typing: The production of DL-A antibodies for serological typing was continued. The typing laboratory participated in the second International Workshop on Canine Immunogenetics in Portland, Oregon, December 1974. The sera produced in Ulm were tested at this workshop in 877 dogs (61 families, including 451 family members and 426 mongrel dogs). A detailed report on the various sera is at present in preparation.

3. Mixed lymphocyte reaction: By means of a semi-micro method developed and applied to family members in various combinations, DL-A-LD specificities were defined. Five homozygous and four heterozygous reference cells can now be used for LD typing.

4. Cell-mediated lympholysis (CML): This technique was adapted for dog lymphocytes. It could be shown that both DL-A-LD and DL-A-SD differences are required for positive reactions, and that SD antigens of both the first and the second DL-A series are target determinants for CML.

5. Further lines of investigation in 1975:
From these data it appears that the mitigation of GvHR is of utmost importance for the further development of the allogeneic stem-cell transfusion model. We try to reach this goal by three procedures:

   1. A treatment regimen of methotrexate will be applied to DL-A identical, MLC negative donor-recipient combinations in our system.
   2. Attempts will be started to purify stem-cells from the peripheral blood by discontinuous albumin gradients and other methods and to test their in vitro immunological and stem-cell qualities. At a later stage, stem-cell fractions will be transplanted into lethally irradiated dogs.
   3. Test systems will be developed for the typing of antigens governed by regions outside the MHC. Donor-recipient combinations could be typed for non-reactivity by a mixed epidermis-lymphocyte reaction.

   It is hoped that these approaches may soon lead to the application of autologous and allogeneic transfusion in man.

Ergebnisse des Projektes Nr. 3

Leiter des Projektes und wissenschaftliche Mitarbeiter:

T. M. Fliedner, M. Dietrich (clinical), H. Heit (experimental) with C. Abt, W. Heit, G. Hochapfel, D. Krieger, H. Meyer, H. Pflieger, H. Rasche, E. Vanek

Titel des Projektes:

Bacterial decontamination as a means of combating bacterial infection and graft-versus-host disease in patients and experimental animals with bone marrow failure and after bone marrow transfusion.

Results:

Clinical Gnotobiology:

In 1974 the randomisation of patients with acute leukemia within the protocol of the EORTC Gnotobiotic Project Group was completed. The Ulm unit has, so far, treated 67 patients under this program that comprises about 150 patients at clinical centers in several countries in Europe. The first aim of this clinical study was to determine the efficacy of strict reverse isolation procedures and decontamination techniques. Preliminary evaluation of the patients' data from the Ulm unit (only a part of the whole study) showed a significant rise in the complete remission rate of acute leukemia and the 30-day survival rate after completion of the observation and treatment phases. Also preliminary suggestions are that the oral cavity is most resistant to decontamination procedures, thus leaving a possibility of endogenous infection by microbes of this local origin. The contamination rate was consistently low in the Ulm setting, indicating that the sterilisation procedures and the isolation techniques that had been developed there are of a standard comparable with other well-known centers.

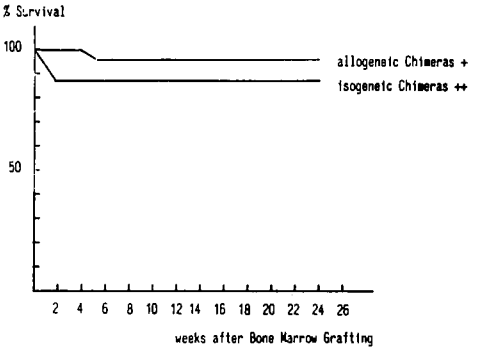
The EORTC study, including the patient data of the Ulm unit, will be fully evaluated during 1975. Meanwhile, a pilot program is in progress to study whether certain antibiotic combinations and new application techniques will result in better decontami-

nation than can be achieved with present methods. With the final evaluation of the prospective randomised study at hand, possibly also supported by suggestions from the pilot study in progress on the improvement of decontamination, further multicentral studies and cooperative programs will be developed in 1975/76 to achieve the final target: that is, germfree conditions and an infection rate close to zero with improvement of the treatment programs, such as cytostatic chemotherapy to induce the maximal remission of acute leukemia, optimal conditions for bone marrow transplantation, bone marrow failure and combined immune deficiencies. Other benign bone marrow failures of short duration with high risk of lethal infection are currently being considered. These leukemia studies provide the necessary basis for the use of this approach for the treatment of radiation-induced hemopoietic failure.

Experimental Gnotobiology

In experimental gnotobiology, the studies during 1974 confirmed our previous notion that bacterial decontamination, induced in mice by means of non-resorbable antibiotics and maintained in a germfree environment, provides animals that show a very low mortality when transplanted with allogeneic bone marrow after 800 rad whole body irradiation. In figure 1,

Fig. 1 Long Term Survival of "allogeneic" and Isogeneic conventional Chimeras - 50 animals each group -



+ allogeneic chimeras were grafted with 10 x 10^6 b.m. - cells

++ isogeneic chimeras were grafted with 1,0 x 10^6 b.m. - cells

the data are given for long-term survival of decontaminated allogeneic and isogeneic conventional bone marrow chimaeras. Chimaerism in the allogeneic animals was proven by means of hemoglobin electrophoresis (recipients: CBA-CA mice, donors: C_{57} Bl mice). Although signs of GvH were observed, the animals recovered. On the basis of several considerations, the data of such experiments lead to the tentative conclusion that both in the bone marrow and in the spleen of long-term chimaeric animals, the lymphocytic population loses most of its potential to react with the same type of histoincompatible antigens when confronted with the primary host. As GvHR is observed to be initiated by the allograft in the first recipients, a humoral "inactivation" by blocking factors seems to be of little relevance. Alternatively, aside from the quantitative aspect of a reduced immunocompetence, one might speculate that, under the experimental conditions described above, the aging of the graft over the period of observation of about 56 weeks may be a limiting factor for the reactivity of the lymphocytes involved.

Finally, it was of interest to observe that the reassociation of the decontaminated allogeneic bone marrow chimaeras with a conventional flora did not result in a increased mortality. The reconventionalized animals survived without any signs of secondary disease, indicating that, after the productive phase, no relapse from secondary disease can be provoked.

Ergebnisse des Projektes Nr. 4

Leiter des Projektes und wissenschaftliche Mitarbeiter:

B. Kubanek with O. Bock, E. Bock, W. Heit and W. Schreml

Titel des Projektes:

Comparative investigations on the damage and repair of hemopoietic cells by ^{3}H-Thymidine or radiomimetic substances with particular reference to the uncommitted and committed stem-cell compartments.

Results:

In 1974, the emphasis of this project was on 1. obtaining further insight into the mechanisms that regulate fetal hemopoiesis and on 2. executing a comparative study of the suicidal effect of tritiated thymidine and of hydroxyurea on the uncommitted and committed stem-cell compartments in the mouse.

Regulation of fetal hemopoiesis:

The recovery of hemopoiesis in fetal liver was investigated after the administration of 1000 mg/kg Hydroxyurea (HU) to CBA mice on the 13th day of gestation. The total cellularity of the fetal liver began to decrease at the 12th hour and reached its nadir at 24 hrs, being then 30 % of the controls. Thereafter, slow recovery occurred and the cellularity of the liver approached control values by the 72nd hour after the injection of HU. A similar recovery pattern was observed for the CFUc which showed an initial reduction of 50 % and a further decrease at the 12th hour. Then, a slow recovery occurred reaching control values by the 96th hour after HU. Erythropoiesis, judged by the erythropoietin response of fetal liver cultures and absolute cell counts of erythropoietic cells, was not above controls before the 72nd hour. The CFUs content of fetal livers reached a minimum of 35 % of the controls at 12 hours, but increased rapidly thereafter in contrast to the

more differentiated cell compartments, reaching control values by the 48th hour with a substantial overshoot at 72 and 96 hours. The calculated doubling time of the CFUs was 12 hours between 12 and 72 hours after HU, compared to 20 hours in the control embryos. The fraction of CFUs in S-phase, estimated by the in vitro ^{3}H-TdR suicide technique, was not significantly different in the two experimental groups, so that the difference in doubling time could have been caused not only by a shortening of the overall generation time but also by a shortening of the DNA synthesis time. From preliminary synchronisation experiments the DNA synthesis time of fetal liver hemopoiesis was estimated to be 5 hours, which appears to be shorter than for adult hemopoiesis. From these results, it was concluded that, after a partial depletion, CFUs self-replication seems to have a higher priority than the restoration of the more differentiated compartment; whereas, in adult mice, differentiation into the committed stem-cell compartments seems to occur before the restoration of the CFUs compartment under similar conditions.

Effects of tritiated thymidine compared to Hydroxyurea.

^{3}H-Thymidine and Hydroxyurea were used to determine the proportion of stem-cells in S-phase. However, marked differences were observed when either 0.8 mCi ^{3}H-TdR or 1000 mg/kg HU was administered to polycythaemic CBA-mice. The initial depression was similar for CFUs and ACUs, but increased for ERCs after ^{3}H-TdR. In ^{3}H-TdR animals, recovery of all stem-cell compartments was depressed, but improved when ^{3}H-TdR was followed by HU 15 min later. To test ^{3}H-TdR reutilisation as a possible mechanism to explain the difference between suicidal ^{3}H-TdR and HU, the fate of tritium activity was followed in animals after suicidal or tracer doses of ^{3}H-TdR in combination with HU. The parameters tested were the specific

activity of DNA and the distribution of activity in DNA and low molecular weight compounds (acid-soluble, not volatile and water-soluble, volatile). ^{125}I-UdR was administered to measure the degree of reutilisation. DNA-bound tritium activity in bone marrow cells decreases to less than 15 % of the 1 h value within 6 h, at which time HU is given (after a tracer or suicidal ^{3}H-TdR dose), while, after ^{3}H-TdR alone, 50 % is still present at 24 h, indicating a more rapid destruction of labelled cells by HU compared to ^{3}H-TdR. After ^{3}H-TdR, the activity in the low molecular weight fraction, where precursors for reutilisation are to be expected, is absolutely and relatively higher after ^{3}H-TdR alone. The divergence of DNA-specific activity for ^{3}H-TdR and ^{125}I-UdR indicates increased reutilisation for all experimental groups compared to control animals. Reutilisation occurs predominantly within the first 24 h after HU and for a much longer period after ^{3}H-TdR. These data suggest that reutilisation enhances the killing efficiency of ^{3}H-TdR on stem-cells and that the addition of HU reduces the degree of reutilisation, thus shifting the killing efficiency of ^{3}H-TdR closer to that of HU.

The results presented give further insight into the functional structure of the hemopoietic stem-cell pool and its cellular composition with respect to the relationship between uncommitted and committed stem-cells. This question is of major importance if one wants to know more about the mechanisms of hemopoietic recovery after whole body radiation exposure and bone marrow transplantation in order to perhaps control and improve the rate of efficient recovery.

Ergebnisse des Projektes Nr. 5

Leiter des Projektes und wissenschaftliche Mitarbeiter:

G. Lucarelli, A. Porcellini, T. Izzi, M. Galimberti, M. Tomasucci, A. Fontebuoni, A. Bravetti and E. Guardato

Titel des Projektes:

Comparative study on the characterization of the fetal and neonatal stem-cell compartments as a basis for studies on the regulation of hemopoietic cell kinetics.

Results:

Studies on fetal liver cell suspension and on fetal liver tissue were done using an "in vivo" culture system provided by the diffusion chamber (DC) method. Fetal liver was cultured in "DC" either as whole organ or as cell suspension of equivalent concentration. Only the whole organ was found to be able to maintain erythroid differentiation during a ten-day culture period. When fetal liver and fetal liver cell suspension were cultured in "DC" implanted in bled mice, host erythropoietin exerted its effect only in the whole organ culture suggesting a cooperative action of erythropoietin microenvironment in inducing erythroid cell differentiation. From studies on fetal hemopoietic tissue, the question arose, whether the stem-cell of fetal liver responds to erythropoietin for erythroid differentation or not. There are several data obtained "in vitro" supporting the hypothesis of erythropoietin-dependent erythropoiesis during fetal life. However, "in vitro" observations appear to be in contrast with several "in vivo" observations and the problem remains, at the moment, open. Data from our studies on neonatal erythropoiesis could retrospectively shed light on the problem of neonatal erythropoiesis in being only partially under humoral regulation. We have suggested as a hypothesis that during the fetal hepatic hemopoietic period there is a shift from a fetal-type stem-cell to an adult-type stem-cell. The fetal-type stem-cell differentiates toward the erythroid compartment independent of erythro-

poietin, while adult-type stem-cell seeding of the bone marrow acquires the capacity to form an erythroid committed cell compartment, whose amplification in response to an augmented peripheral request for red blood cell production is regulated by erythropoietin. While migrating from liver to bone marrow, the stem cell acquires an active genetic locus sensitive to the derepressor effect of erythropoietin while in the immature fetal-type stem-cell, the receptor for hemoglobin synthesis is derepressed by factors other than erythropoietin. (This hypothesis has been submitted to Lancet for publication). For the purpose of studying the validity of the above hypothesis, studies have been continued on the newborn rat using a statokinetic agent (colchicine) and a DNA-blocking agent (HU). It has been observed that, in the newborn rat, efflux from the stem-cell compartment to the erythroid compartment continues during starvation or hypertransfusion despite disappearance of erythropoietin in the starved, newborn rat. Newly-formed erythroblasts continue to appear during starvation but the transit time of erythroid cells is extremely prolonged from the normal 16 hours to 60 hours, with a time for mitosis of 4 hours compared to the normal 1 hour. These have been studied using the in vivo culture system for bone marrow from the newborn rat at various ages and preliminary data show that in this system neonatal bone marrow rapidly gives rise to a population of differentiated granulocytes while erythropoiesis appears to be scarcely represented.

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Contractant van de Commissie	: Nederlandse Organisatie voor Toegepast Natuurwetenschappelijk Onderzoek TNO
Nummer van het contract	: 088-72-1-BIAC
Hoofd van de researchteams	: Prof. D.W. van Bekkum
Algemeen onderwerp van het contract	: Consequences of radiation exposure, prevention and treatment of pathological effects

A. General description of program :

Histocompatibility largely determines the severity of the Graft-versus-Host (GvH) reaction in recipients of allogeneic bone marrow. Thus, the primate studies in 1974 were again centered on improving methods of selecting the most compatible host/donor combinations among unrelated rhesus monkeys.

Until recently, "matching" depended on the serologically defined (SD) antigens only. The majority of the SD antigens of RhL-A (the monkey's major histocompatibility complex or MHC) is now well defined and matching for SD is fast and relatively simple. Other relevant factors for matching are the lymphocyte defined (LD) determinants, responsible for mixed lymphocyte reactivity (MLR) in vitro. Matching for LD is less reliable and, in general, still depending on time-consuming cell cultures. Therefore, efforts are made in man, rhesus monkey and dog, to identify the LD-determinants by more direct methods, using "typing cells" and specific antisera. In 1974 considerable progress was made in collecting appropriate LD-typing cells but seriological typing for LD determinants is still in its beginnings, in any species. Finally, there is a third and new class of antigens,which, in the mouse at least, is relevant to histocompatibility, especially with regard to GvH reactions: the Ia or "immune-region-associated" antigens of the MHC. Antigens of Ia characteristics have been identified also in the rhesus monkey. They are somehow associated with LD or MLR determinants but their exact relation is not yet known, for any species.

Thus, in selecting histocompatible unrelated donors for bone marrow grafting, 3 classes of antigens now have to be taken into account: conventional SD antigens, LD or MLR antigens and the still rather enigmatic Ia antigens.

Experiments with grafts of purified stem cell concentrates from random allogeneic donors into irradiated monkeys were continued to evaluate the mitigating influence of total bacteriological decontamination on the severity of the GvH reaction. In irradiated dogs the studies were directed at defining conditions for obtaining a take of random allogeneic bone marrow grafts using moderate numbers of bone marrow cells which are practicable in human patients. Furthermore, factors determining the occurrence of GvH reactions in compatible sibling donor-host combinations were explored in order to arrive at a predictive test for GvH in this situation.

In the mouse system the work has centered on defining the properties of fetal liver haemopoietic stem cells and to evaluate the possibility of employing fetal stem cells as an alternative for adult bone marrow cells in human bone marrow transplantation.

Resultaten van het projekt No. 1

Hoofd van het team en wetenschappelijke medewerkers : D.W. van Bekkum, H. Balner, K.A. Dicke, B. Löwenberg, H.M. Vriesendorp.

Titel van het projekt : Bone marrow transplantation

B. Results in 1974

For the evaluation of matching procedures in monkeys, simple skin grafting experiments usually precede bone marrow transplantation. In 1974 it was shown that matching unrelated host/donor combinations for SD antigens only, significantly improved skin graft survival times. On the other hand, MLR (or LD) compatibility empirically determined by nonreactivity of recipient lymphocytes against mitomycin-treated donor cells, did not influence graft survival. These results are in disagreement with our own impressions from pilot experiments in monkeys in 1973 and with preliminary skin graft data obtained by others in human volunteers. Similar selection procedures are now applied to establish whether SD identity (with or without MLR non-reactivity) influences kidney graft survival and GvH reactions in unrelated rhesus monkeys. The latter results should influence the decision whether or not to attempt human bone marrow grafting based on such limited matching (naturally in combination with other means to mitigate GvH !).

In the meantime, improved methods of matching for LD antigens (see general description) and for the postulated Ia antigens, may increase our chances of selecting compatible unrelated donors for marrow transplantation. Monkey experiments of this kind are anticipated for late 1975 or early 1976.

Studies with related rhesus monkeys will also be continued. A small breeding colony has been earmarked for the bone marrow transplantation program so that sibs and half-sibs sharing 0, 1 or 2 RhL-A haplotypes should be available for marrow grafting within the coming years.

Dog bone marrow grafting was continued in two directions :

a) The only donor-recipient combinations which are acceptable for bone marrow grafting in humans are monozygotic twins and sibs identical for the Major Histocompatibility Complex (MHC), which leaves appr. 70% of the patients who could benefit from a bone marrow graft, without a donor. For that reason an exploration was started of the feasibility of the use of donors not compatible for MHC.

A take of MHC nonidentical bone marrow is more difficult to achieve than one of MHC identical bone marrow. This genetically determined phenomenon named allogeneic resistance (AR), was found to be controlled in dogs by a genetic system or systems (labelled R of resistance). R. is closely linked to DL-A (the MHC of the dog), but different from SD and LD systems. AR could be overcome by i.v. injections of silica particles. Studies of GvH treatment and prevention of MHC nonidentical bone marrow have now been started in dogs, using moderate bone marrow cell numbers.

b) GvH reactions occur in appr. 50% of the MHC identical sibs. Half of these GvH reactions are fatal. Experiments aimed at identifying factors which will predict the MHC identical donor-recipient pairs which will develop GvH, were performed.

The results indicate that the locus for the polymorphic red cell enzyme P4M2 is probably linked to a minor histocompatibility locus, controlling GvH reactions in DL-A identical sibs. The MLC third party ratio (according to Park and Good) appeared to be less predictive for GvH reactions. A next series of experiments will include new genetic tests and reagents for a better identification of the genetic system(s) causing GvH disease in DL-A identicals. Genetic studies of GvH disease in DL-A mismatched animals were also started in an attempt to define in more detail which part of the DL-A complex is controlling GvH reactions.

Transplantation experiments with fetal liver stem cells in mice have yielded support for the assumption that the fetal liver haemopoietic stem cell (HSC) is distinct from the adult bone marrow HSC in that the former has a higher rate of multiplication post transplantation and induces less delayed type GvH than the adult HSC. However, the takeability of allogeneic fetal HSC is less favourable than that of adult HSC, which limits the applicability of fetal liver cell transplants because of lack of cells. First attempts to produce larger numbers of fetal HSC by in vitro culture were encouraging in that factors were found that induce proliferation of stem cells in the culture system.

Total bacteriological decontamination and isolation was shown to prevent completely mortality due to the delayed type of GvH disease in mice following allogeneic bone marrow transplantation. Reconventionalization as early as 40 days following grafting could be performed without recurrence of GvH disease. In monkeys, receiving purified bone marrow stem cell concentrates, delayed type GvH disease was effectively prevented by total decontamination, but the monkeys subsequently died from endogenous systemic virus infections. The latter complication is likely to be associated with severe immune deficiency and new approaches have to be devised to promote the recovery of the immune system in those radiation chimeras.

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Contractant de la Commission : Prof. TAGNON

N° du contrat : 088 BIAC

Chef du (des) groupe(s) de recherche : Dr. STRYCKMANS

Thème général du contrat : Kinetics and regulation of cell proliferation of normal and pathological bone marrow cells.

I. In order to protect the human bone marrow from the lethal effect of external agents, it is essential to recognize which cells in the bone marrow are the target of a regulation feedback mechnism and which ones are the most sensitive to the effect of external chemical agents.

The proliferating myeloid recognizable precursors in different stages of their maturation toward the mature polymorphonuclear end cell, were examined for their eventual differences in sensitivity. Therefore, the myeloid cells were examined for their response to an hypothetical endogenous humoral agent probably regulating their proliferation. These cells were also examined for their sensitivity to a well known myelotoxic agent extensively used in the treatment of leukemia ; namely cytosine arabinoside.

Human bone marrow cells were examined on autoradiographies after their labeling with ^{3}H thymidine. The cells were either "flash" labeled in vitro in order to estimate their proliferative activity or labeled in vivo in order to follow their fate and their progeny in the peripheral blood.

The present work on human bone marrow indicates that the proliferating myeloid cells probably corresponding to those performing the last 2 - 3 divisions were less sensitive than more immature myeloid cells to feedback regulation on one hand and cytosine arabinoside on the other hand.

II. In order to protect patients with bone marrow aplasia from severe and often lethal, infections, granulocyte transfusions have been performed during the last decade by several groups. The validity of this procedure has never been clearly established in humans so far. This problem has been investigated in the second part of this work.

Résultats du projet n° 088-BIAC

Chef du projet et collaborateurs scientifiques : P. Stryckmans, L. Debusscher, E. Collard-Rongé et D. Gangji.

Titre du projet : Study of the sensitivity of proliferating myeloid bone marrow cells to regulatory mechanism and external chemical agents as a function of their differentiation.

1.- Regulatory mechanisms

A decreased proliferative activity of the myeloblasts in the bone marrow of patients with acute myeloblastic leukemia (AML), at the time of diagnosis when the number of myeloblasts in the body is high, is well known. We have demonstrated that this proliferative activity returns to normal values when the patients are in complete remission of their disease i.e. with a low number of myeloblasts is obtained as a consequence of the treatment. This, however, cannot be interpreted as the consequence of a regulatory feedback mechanism in vue of the uncertainties relative to the leukemic or non-leukemic nature of the myeloblast seen in remission of AML.

In chronic myeloid leukemia (CML) the persistance of the Philadelphia chromosome abnormality is observed in the bone marrow cells all along the evolution of the disease. This makes it certain that one is dealing with the same pathological cell line whether one examines the bone marrow in relapse or in remission. The proliferative activity of the myeloblasts in the bone marrow of patients with CML was found to be decreased in the active phase of the disease (more than 40,000 WBC/mm^3). The mean labeling index with ^{3}H-thymidine ($\sim$ 42 %) was identical to the mean value obtained in hematologically normal individuals when a remission (less than 20,000 WBC/mm^3) was obtained by chemotherapy. The difference was highly significant ($P < 0.01$). The following factors were examined as possible explanations for the results : (1) the re-emergence of normal Philadelphia-negative cells during remission ; (2) variations of the DNA synthesis time with the peripheral blood leukocyte count ; (3) the contamination of the marrow samples by less proliferating blood myeloblasts, and (4) a direct effect of the previous treatment on the myeloblast proliferation. All these factors could be ruled out. It is concluded therefore that myeloblasts in CML remain sensitive to a regulatory feedback mechanism probably operating on normal myeloblasts.

On the other hand, no significant difference was found between the proliferative activity of myelocytes from hematologically normal individuals, from CML patients in relapse and from CML patients in remission . Therefore,

at least in CML, more mature cells such as myelocytes appear to be no longer responding to regulatory mechanisms affecting the myeloblasts.

Our results on the response of the myeloblasts seem to be identical to the one described by Moore et al. (JNCI, 1973) for "colony-forming cells" i.e. myeloid cells more immature than the myeloblasts.

2.- Chemotherapy.

Cytosine arabinoside, an antipyrimidine agent used for the treatment of leukemia, was found in humans (at the dose of 600 mg/m^2 BSA) to be more toxic for the very immature myeloid cells (stem cell ? colony-forming cell ?) than for more mature and differentiated recognizable proliferating myeloid cells (promyelocytes, myelocytes).

These results were obtained by labeling the proliferating myeloid cells in vivo by ^{3}H-thymidine and by following in the blood their labeled progeny namely the mature polymorphonuclear cells (PMN). It was found that only the PMN released from the bone marrow in the blood more than 10 days after the injection of the drug were considerably reduced in number (less than 5 % of the pretreatment level). On the other hand, before the 10^{th} day, the number of blood PMN was only slightly decreased. This indicates that the more mature proliferating myeloid cells in the bone marrow are less affected by the drug than less differentiated cells (stem cells ? colony-forming cells ?). Whether this is related to differences in the proliferative activity between the mature recognizable myeloid cells in the bone marrow and the less differentiated myeloid precursors of the bone marrow cannot be ascertained by the present study.

Résultats du projet n° 088-BIAC

Chef du projet et collaborateurs scientifiques : P. Stryckmans, L. Debusscher, E. Collard-Rongé et D. Gangji.

Titre du projet : White blood cell transfusions to neutropenic patients with bone marrow aplasia.

Thirty patients with severe neutropenia (500 polymorphonuclear cells PMN/mm^3) consecutive to bone marrow aplasia (toxic, leukemic or idiopathic) were studied for the evaluation of white cell transfusions as and adjuvant to adequate antibiotherapy in the treatment of infection. The indication of white cells transfusions was defined as temperature 38.5° C and/or clinical evidence of infection.

The white cells were collected from normal volunteer donors by the procedure of continuous flow centrifugation using the NCI/IBM Blood Cell Separator. Thirty-three neutropenic patients at the onset of infection were randomly assigned to either antibiotherapy alone or antibiotherapy plus white cells transfusions (average of 10^{10} PMN per transfusion) on 2 consecutive days within the 72 hours following the onset of infection.

Out of 33 patients investigated, only 22 were found retrospectively to be evaluable since no evidence for a bacteriological infection was obtained a posteriori in the other 11 patients. Out of these 22 patients, 11 had received transfusions, the other 11 patients had not. The recognized prognostic factors were found to be equally distributed in the two groups which were thus considered as comparable groups.

No significant difference of infectious morbidity was found between the two groups. The mortality of these high risk patients was zero in both groups indicating that early and adequate antibiotherapy is very efficient and that the demonstration of the eventual efficacy of PMN transfusion in infected neutropenic patients will need very large strictly controlled studies.

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Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung,
Institut für Hämatologie

Nr. des Vertrages: 089-72-1 BIAD

Leiter der Forschungsgruppe: Priv.-Doz.Dr.S.Thierfelder

Allgemeines Thema des Vertrages:

Strahlenbiologische Hämatologie und Immunologie
(Proj. 1-3)

(Proj. 4-7 über Nuklearmedizinische Hämatologie sind unter Kapitel V "Forschungstätigkeit Anwendungen Medizin" aufgeführt. Am Schluß dieses Teils des Berichtes befindet sich eine Aufstellung der Publikationen.)

Allgemeine Darstellung der durchgeführten Arbeiten:

The projects of the research group in Munich continued their studies on the analysis and treatment of the consequences of radiation exposure. The principal effort was concerned with the treatment of bone marrow failure following total body irradiation or high doses of cytostatic drugs used in anti-cancer therapy. Experiments in rodents as well as preclinical and/or in vivo studies in dogs and men were undertaken to overcome the immunological complications of bone marrow transplantation in radiation or drug-induced hemopoietic failure. They were concentrated on two research areas: immune-intervention with specific anti-T-cell sera and histocompatibility typing to prevent secondary disease. While the first approach is being studied extensively in our group, collaborative research in histocompatibility typing became particularily fruitful on the European level. The 3rd meeting of the Cooperative Group on Bone Marrow Transplantation in Dogs was held in TNO Rijswijk.

A joint project was set up by the participating groups from Holland, Ulm, Paris and Munich to study the long-term histologic, immunologic and hematologic consequences of marrow transplantated dogs with the member laboratories taking advantage of the animal housing facilities for dogs in our GSF. In rodents successful immune-intervention led to the complete suppression of acute secondary disease. But also chronic secondary disease responded to this treatment in several H_2 incompatible donor-recipient combinations. An anti-T-cell serum developed against human thymocytes was likewise found to kill T-cells and spare colony forming cells. In dogs collaborative histocompatibility typing for LD in Munich and TNO Rijswijk led to the definition of the MLC-specificities DL 50-DL 57. In men 44% of the HL-A-Haplotypes 3-7 were found to carry the MLC-specificity Pi and 46% of the HL-A-Haplotypes 3-W5 had the MLC-specificity Pf. Our development of a technique to store lymphocytes for MLC at low temperatures will facilitate the definition of the MLC-system in dogs and men. The transplantation of SD-LD-heterozygous canine marrow into SD-LD-homozygous recipients conditioned with cyclophosphamide permitted the study of host-versus-graft activity. Histologic studies on lymphnodes from dog chimaeras were performed in the Abteilung für allgemeine und experimentelle Pathologie.

Successful treatment of bone marrow failure depends also on further advances in the diagnosis of the underlying disease. Therefore another project concerns itself with the proliferation kinetics of erythropoiesis in various types of anemias calculating DNA synthesis and the ^{3}H-TdR labeling index. Intramedullary cell-death or disappearance of precursor cells in certain stages of differentiation could be objectivated by this approach in pancytopenias. An extensive ineffective erythropoiesis restricted to the more mature cell type was found in thalassemia major.

Refined methods of hemato-morphology such as bone marrow biopsies were applied to a study on the long-term consequences of ^{32}P in polycythemia vera showing reduced hemopoiesis and marrow sinuses.

Ergebnisse des Projekts Nr. 1 und 2 (zusammengelegt)

Leiter des Projekts und wissenschaftliche Mitarbeiter:
S. Thierfelder, H. Kolb und H. Rodt

Titel des Projekts:
Partial body irradiation and other non-lethal conditioning treatments of bone marrow recipients.

This project on rodents was combined with the project No. 2 on dogs dealing also with conditioning of marrow recipients. A study on the suppression of host-versus-graft reactivity after radiation following a treatment with ALS was undertaken. Using the lymphnode weight assay a residual host-versus-graft reactivity could be demonstrated after as high a dose of total body irradiation as 1100 R (^{137}Cs, 60R/min). Whether this activity stems from relatively radiation-resistant cells such as macrophages is under investigation. A three day's treatment of ALS 0,25ml/day completely suppressed host-versus-graft reactions after supralethal radiation. The combination of ALS and radiation decreases the minimal dose of radiation necessary to condition bone marrow recipients as demonstrated by us in the last report.
In dogs conditioning regimens using cyclophosphamide were studied. A lethal dose of 3 x 40 mg/kg divided over 3 days conditioned recipients for DL-A compatible marrow. The addition of donor lymphocytes increased the lymphatic chimaerism. Histologic studies on dog chimaeras conditioned with cyclophosphamide showed a normal lymphnode structure as early as 50 days after transplantation. SD-LD-homozygous canine recipients of SD-LD-heterozygous bone marrow showed less secondary disease but often reversion to recipient type hemopoiesis due probably to comparatively stronger host-versus-graft reactivity. The reverse donor-recipient combination led to severe secondary disease.

The studies in mice and dogs clearly show that host-versus-graft reactivity cannot be suppressed completely by total body irradiation or cyclophosphamide in the more incompatible donor-recipient combinations. Additional measures like ALS directed against possibly more radiation- or drug-resistant cells must be added to the conditioning regimen.

Ergebnisse des Projekts Nr. 3

Leiter des Projekts und wissenschaftliche Mitarbeiter:
R. Burkhardt, E. Beil und A. Kronseder

Titel des Projekts:
Bone marrow histology in patients treated with radiation, isotopes and radiomimetic agents.

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1. Due to technical reasons on the side of our clinical colleagues, the investigation of bone marrow changes after local gamma-irradiation had to be interrupted for a one year's period.
During the next year the investigations will be continued with the control of the late changes after intervals of at least 12 months from the last radiation dose. This project seems to be promising according to the observation of a decrease of the endothelial functions of the marrow for years after therapeutic irradiation of the pelvic bones (Bell,1969).

2. In pursuit of our study of the long-term consequences of therapeutic admission of 32^{P} in polycythemia vera, 29 new marrow biopsies could be added, 11 of which belonged to previously controlled cases. In each case, the clinical and biochemical data have been collected together with the histologic observations from serial sections of each sample, comprising at least 5 different stains. Preliminary results have been reported in an invited lecture on the Pathological Institute of the University of Leiden (Netherlands) in July 1974. The leaping point is the complete lack of evidence of an acceleration of secondary marrow fibrosis, whilst the number of the marrow sinuses is reduced together with the hematopoiesis including the megakaryocytes. Part of these results has been cited in the following publications: R. Burkhardt, Therapy of the myeloproliferative Syndromes, in: Therapie Innerer Krankheiten, herausgegeben von E. Buchborn et al., Springer-Verlag Berlin-Heidelberg-New York, 1973, and in: MOS-Syndrome, Review of Literature and Histomorphology, Dahlem-Konferenz 1974, in: Myelofibrosis - Osteomyelosclerosis Syndromes,

ed. by R. Good, Pergamon Press, Berlin, 1975, in press.
This study should be continued for a period of about another 2 or 3 years, to secure sufficient statistical evidence for the different histological signs under observation, e.g. morphometric changes of bone parameters, cellularity of the marrow, number and changes of the blood vessels, and changes of the stromal tissues.

Associato della Commissione: Comitato Nazionale per l'Energia Nucleare, Laboratorio di Radiobiologia Animale

N° del contratto: 108-72-1 BIOI

Capo del Gruppo di ricerca: prof. G. Doria

Tema generale del contratto: Protezione e riparazione del sistema immunitario del danno delle radiazioni.

Collaboratori scientifici: dott. G. Agarossi, dott. G. Gorini.

The immune system protects the individual from invasion and pathogenic action of microorganisms. Efficiency of the immunologic surveillance relies on integrity of cell populations that participate in the processes of antigen recognition and antibody production. Several immune responses require the cooperation of macrophages, thymus-derived (T), and bone marrow-derived (B) lymphocytes, which results in antibody production by B cells.

The protective ability of the immune system ultimately depends on the good fit of antibodies for antigens. Indeed, neutralization of viruses or toxins is influenced by antibody avidity, a function of affinity, which determines the stability of the antigen-antibody complexes. Affinity of serum antibodies increases with time after immunization and with lowering the antigen dose. Variations in antibody affinity are attributed to changes in selective pressure exerted by antigen concentration at the level of the precursors of antibody-producing cells. These precursor B cells possess membrane-bound receptors with a wide range of affinities while the receptors of each precursor cell seem to have the same affinity as the antibody secreted upon the combination of antigen with the receptors. Thus, the precursors of B cells producing antibodies with low affinity need higher concentration of antigen to be triggered to antibody formation than the precursors of B cells producing high affinity antibodies. As antigen concentration decreases with time after immunization, the precursors of cells producing higher affinity antibodies are selectively stimulated, a change in the lymphoid

cell population that is delayed by relatively large doses of antigen. Experimental evidence from antibody responses to carrier-hapten conjugates indicates that carrier stimulation of T cells favours selection of B cells producing high affinity antibodies against the hapten.

It is well known that the immune system is highly sensitive to radiation, as the lymphatic damage caused by sublethal doses of X-rays results in impairment of antibody production. While there is little doubt that B lymphocytes are extremely radiosensitive, it is as yet unclear whether macrophages and T lymphocytes should be considered as radiosensitive. These uncertainties rely mainly on the irradiation conditions used (in vivo or in vitro) and on the cellular functions investigated. In several animal species spontaneous recovery of the immune system is complete within 2 months from exposure to large sublethal doses of X-rays. Recovery of the immune system after supralethal doses of X-rays can be achieved by bone marrow transplantation. The normal level of immune response may be observed within 2 months from transplantation of syngenic cells, whereas it is not yet attained within 10 months from transplantation of allogenic or xenogenic cells.

Since in none of the previous studies on the recovery of the immune system after sublethal or supralethal doses of X-rays had antibody affinity been evaluated, experiments were carried out to investigate the recovery of the immune response to the hapten-carrier conjugate DNP-KLH (dinitrophenyl--keyhole limpet hemocyanin) under several experimental conditions by estimating the concentration and affinity of serum antibodies specific for DNP. Both measurements were performed by the equilibrium dialysis technique whereby immunoglobulins prepared by serum precipitation with 40% ammonium sulfate were reacted against several concentrations of tritium-labelled DNP-lysine. From binding data at equilibrium the antibody concentration was estimated as moles of total combining sites per liter of serum (M/L) and the antibody affinity as the reciprocal of the DNP-lysine concentration(L/M) at which 50% of the combining sites are saturated.

Recovery after a sublethal dose of X-rays. C3H mice received a total body dose of 450 R and, either immediately (group A) or 30 days later (group B), a subcutaneous injection of 0.1 mg of DNP-KLH in Freund complete adjuvant. After 10, 20, 30, or 60 days from antigen injection 10 mice of each group were injected again subcutaneously with 0.1 mg of DNP-KLH in solution, bled 7 days later, and their sera pooled for determination of antibody concentration and affinity. Unirradiated C3H mice were immunized and bled as above and served as controls (group C).

The rate of antibody production was found lower in group A than in C, whereas it was the same in groups B and C. The data show that when the animals were primed immediately after irradiation the secondary response to antigen given at day 10 yielded an antibody concentration 20 fold lower than that in unirradiated controls. This difference in antibody concentration decreased sharply when the second antigen was given at day 20 or 30 and vanished at day 60. When the animals were primed a month after irradiation, the secondary response was always comparable to that in normal controls, suggesting that full recovery of the immune system occurred within 30 days from exposure to 450 R.

Antibody affinity was found to increase at the same rate in groups A and C, but at higher rate in group B in which affinity values were always above those in groups A and C. Thus, when the irradiated immune system was allowed to recover for 30 days before priming the secondary response to antigen given after 10, 20, 30, or 60 days resulted in normal levels of antibody concentration but in higher affinity than that in unirradiated controls. When the animals were primed immediately after irradiation antibody affinity was the same as in normal

controls although the antibody concentration were subnormal. It can be concluded that recovery of the immune system after a sublethal dose of X-rays results in cell populations from which high affinity B cells are more easily selected by antigen.

Recovery after a supralethal dose of X-rays. Preliminary experiments carried out in our laboratory on the recovery of the immune system in radiation chimeras were repeated. C3H or DBA/2 mice received a total body dose of 900 R and $1x10^7$ C3H or DBA/2 nucleated bone marrow cells in syngenic or allogenic host-donor combination. Four months later all chimeras were injected subcutaneously with 0.1 mg of DNP-KLH in Freund complete adjuvant. After 20, 30, or 60 days, 10 chimeras of each type were injected again subcutaneously with 0.1 mg of DNP-KLH in solution, bled 7 days later and their sera pooled for determination of antibody concentration and affinity. Unirradiated C3H and DBA/2 mice were immunized and bled as above and served as normal controls.

Our previous results were confirmed. Antibody concentration was found much lower in allogenic than in syngenic chimeras, in which values were subnormal at all immunization times. When the second injection of antigen was given on day 30 or 60, antibody affinity was higher in chimeras than in normal controls, while there was no substantial difference between syngenic and allogenic chimeras. Thus, antigen selection of high affinity B cells is more pronounced in radiation chimeras than in normal animals.

The results from both studies on the recovery after a sublethal or supralethal dose of X-rays indicate that spontaneous or artificial (by bone marrow transplantation) repopulation of

the immune system favours the production of high affinity antibodies. This enhanced ability to produce antibodies of high affinity may be very important to assure the survival of those animals with subnormal numbers of antibody-producing cells. Monitoring of macrophage, T, and B cell populations during recovery from radiation damage should be most relevant to identify the cellular changes that provide the immune system with greater sensitivity to antigen selection for high affinity B cells.

PUBLICATIONS APPEARED IN 1974

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Contractant de la Commission :
Université Libre de Bruxelles

N° du contrat : 093-72-1-BIOB

Chef du (des) groupe(s) de recherche :
Jacques E. DUMONT

Thème général du contrat :
Definition of the methodology for the study of the effects of radiation on human tissues (blood, cells, etc.) and application of this methodology.

The general aim of the project is the study of physiological and biochemical mechanisms, the alteration of which causes the short and long term effects of radiation and to develop the methodologies necessary to investigate these mechanisms.

The problems studied are the regulation of erythropoiesis, polymorphonuclear phagocytosis and the development of a mathematical model of follicular cell irradiation by radioisotopes of iodine.

A. Erythropoietin

Irradiation affects erythropoiesis and the catabolism of erythropoietin. The main results obtained in 1974 were :

1) It has been demonstrated that the decrease of erythropoietin catabolism observed in irradiated animals is not a consequence of medullary aplasia.
2) The role of the kidney parenchyma in erythropoietin catabolism has been demonstrated. The existence of a proerythropoietin which could be activated by the kidney is suggested.
3) Androgens, which are potent activators of erythropoiesis fail to act in anephric patients.

B. Polymorphonuclear phagocytosis

Irradiation causes both a leukopenia and a decreased bactericidal activity of the leucocytes. The main results obtained in 1974 were :

1) The killing of bacteria by phagocytosis and iodination is often but not always decreased in irradiated guinea pigs.
2) Evidence is provided that Ca^{++} may be one of the intracellular signals of the metabolic concomitants of phagocytosis.
3) A new form of chronic granulomatous disease is suggested.

C. Model of thyroid follicular cell irradiation

This program is carried out in another Contract.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques :
J.P. NAETS, M. WITTEK, C. DELCROIX

Titre du projet :
Effect of irradiation on the regulation of erythropoiesis

Two effects of irradiation on hematopoiesis are known : a general depression of hematopoiesis and a decrease in the catabolic rate of erythropoietin (Proceed. Soc. Exptl. Biol. Med., 10, 40, 1959). Two aspects of this problem are studied : the control of erythropoiesis and the metabolism of erythropoietin.

A. Erythropoietin metabolism

We have shown that irradiation increases the half life of exogenous and endogenous erythropoietin, i.e., that it slows down its catabolism. After irradiation with 200, 400 or 600r, the half life increased from 1.5h to 2.3h. This effect could be due to medullary aplasia, i.e., to the disappearance of the target tissue, which would then be involved in the catabolism of its regulatory hormone. The definition of dose-response relationship did not allow to reject this hypothesis. However, we have now demonstrated a normal half life of erythropoietin in mice in which medullary aplasia have been induced by the alkylating agent mustine. The action of X rays on erythropoietin catabolism is therefore independent of the medullary aplasia that they cause.

The role of kidney in erythropoietin metabolism has been further investigated. We have shown that erythropoietin half life is prolonged (to 10h) in the anephric animal and demonstrated that this effect is due to the absence of kidney parenchyma rather than to anuria. To better define the role of kidney parenchyma, we have adapted the in vitro dog kidney perfusion system of Nizet (Liège). Erythropoietin has been extracted from the urine of severely anemic patients. Two phenomena have been observed :

a) decrease of erythropoietin activity (half life 2.5h) ;
b) in one case, an increase of erythropoietin activity during the first hour of incubation.

The latter observation suggests that apart from its catabolic role, the kidney could transform a precursor of erythropoietin. Three peaks of protein have been detected by sephadex chromatography of this extract ; only one of which has erythropoietin activity. We are now investigating the hypothesis that one of the two other peaks could contain a proerythropoietin.

B. Control of erythropoiesis

Erythropoiesis, as evaluated by the amount of transfused blood which is necessary to keep up their hematocrit, is much reduced in patients with complete renal failure and even more in anephric patients. We have suggested that in the former case the little renal parenchyma left still provides some erythropoietin.

In order to clarify the mode of action of androgens on erythropoiesis and to reduce the amount of blood required by such patients, the effect of androgens on such patients has been studied. In 10 anephric patients investigated under or without androgen treatment, measurable levels of erythropoietin (i.e., higher than normal) were equally frequent and blood requirements similar. The study is now pursued on patients with some parenchyma left. A positive result would identify kidney parenchyma as the locus of action of androgens.

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The role of the kidney in the catabolism of erythropoietin in the rat.
European Society for Clinical Investigation, Eight Annual Meeting, Rotterdam, April 1974, p. 131 (abstract).

Résultats du projet n° 2

Chef du projet et collaborateurs scientifiques :
E. SCHELL-FREDERICK, R. PARIDAENS, J. VAN SANDE

Titre du projet :
Polymorphonuclear phagocytosis.

Leucocyte function as well as number is critical for the defence of the organism against infection. Our work has been concerned with the mechanisms of uptake and killing of bacteria in normal, diseased and irradiated animal and human polymorphonuclear leucocytes (PMN).

A. Irradiation

In vitro irradiation of guinea pig PMN with doses up to 50,000r has no significant effect on their function. However, leucocytes isolated from animals irradiated in vivo demonstrate decreased phagocytic function prior to the development of leucopenia (Nature, 210, 158, 1966). Evaluation of phagocytic function might represent a more sensitive test for low dose radiation damage. We have attempted to measure the overall activity of the myeloperoxidase enzyme system, which appears to be important for the killing of phagocytized bacteria, in peritoneal exudate PMN from normal and irradiated guinea pigs by the method of iodination (New Engl. J. Med., 284, 744, 1971).

As far, we have demonstrated, but not consistently, a decrease in iodination in resting or phagocyting PMN from irradiated animals. This lack of consistancy appears to be due, at least in part, to variability in the response to irradiation of the guinea pigs used.

B. Cellular mechanism of phagocytosis

The evidence available suggests strongly that the metabolic counterparts of phagocytosis (including the killing mechanisms) are the result of a signal or signals generated by the contact between the phagocytic particle and the cell surface. We have investigated the possibility that Ca^{++} may be such a signal making use of the divalent cation-specific ionophore A23187. This antibiotic appears to act as a freely mobile carrier to equilibrate divalent cation concentrations across membranes via an electroneutral process (J. Biol. Chem., 247, 6970, 1972 ; Arch. Biochem. Biophys., 162, 174, 1974). To prove that Ca^{++} acts as a signal in the phagocytic process one must be able to show :

1) that induction of calcium movement, in the absence of phagocytic particles, can mimic the metabolic changes ;
2) that phagocytosis is associated with changes calcium flux.

We have shown that at external calcium concentrations of 10^{-4}M or higher, the production of calcium flux by A23187 (10^{-6} - 10^{-5}M) reproduces the stimulated oxidative activities characteristic of phagocytosis, i.e., increased oxygen consumption, increased activity of the hexose monophosphate shunt and increased

iodination. The ionophore stimulated oxygen consumption rapidly, within 1 minute. Taking into account the intracellular concentration of calcium ion, 10^{-8} to 10^{-5}M, these results suggest that the observed effects on oxidative metabolism resulted from increased calcium transport across the cell surface membrane into the cell.

In collaboration with Drs. A. Sanfeld and A. Sanfeld-Steinchen of the Faculty of Sciences, U.L.B., work has begun on the elaboration of a model of phagocytosis based on the principles of surface chemistry and hydrodynamics and the experimental data available.

C. Human leucocyte dysfunction

In view of the parallelism between leucocyte dysfunction in irradiated animals and in patients with chronic granulomatous disease, patients with this disease are investigated as models of human irradiated patients.

Studies have been initiated in two siblings,boy and girl, with increased susceptibility to infection. Biochemical studied on their leucocytes demonstrate the defects characteristic of chronic granulomatous disease (CGD). However, the expression of these defects in the leucocytes of the female indicates a different primary enzyme deficiency than that found in the classical X-linked syndrome and is thus of heightened scientific interest.

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LANGZEITWIRKUNGEN UND TOXIKOLOGIE DER RADIOAKTIVEN ELEMENTE

LONG-TERM EFFECTS AND TOXICOLOGY OF RADIOACTIVE ELEMENTS

EFFETS A LONG TERME ET TOXICOLOGIE DES ELEMENTS RADIOACTIFS

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

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

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

096-BIOB Univ. Louvain (Goffeau)

Biology Group Ispra

Contractant de la Commission :
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE - MOL

N° du contrat : 095-72-1-BIOB

Chef des groupes de recherche : Jean R. MAISIN

Thème général du contrat : PROGRAMME DE RECHERCHES AYANT POUR OBJET LES EFFETS A COURT ET A LONG TERME DES RAYONNEMENTS.

The research performed on this contract have been devoted to the following problems :

SHORT TERM EFFECTS

1. The development of biochemical indicators of radiation damage.
2. Effects of X-irradiation and radiomimetic substances on the synthesis of ribosomal and messenger RNA's and on the structure and formation of polyribosomes.

LONG TERM EFFECTS

1. Influence of chemical radioprotectors on the long term effects of ionizing radiation.
2. Studies on biochemical parameters in different organs.

GENETIC EFFECTS

1. Study of the chromosome rearrangements induced in male mice by ionizing radiations.
2. Study of the chromosome rearrangements induced in female germ cells by ionizing radiations.
3. Study of radioinduced chromosome aberrations by banding pattern techniques.

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques :
G. GERBER

Titre du projet : BIOCHEMICAL INDICATORS OF RADIATION DAMAGE

Experiments testing different biochemical parameters in urine for their utility to assess radiation injury were continued. A total of 30 different tests were performed in rats exposed to doses of 100, 200, 300, 400, 600, 800, 1000, 1500 and 2000 R of whole body irradiation. Deoxycytidine, creatine and taurine excretion are the most sensitive indicators of radiation damage, but do not show any further increase at higher doses. Uric acid, sialic acid, RNAse, β-glucuronidase react also after higher doses. A lab manual which is now in print has been prepared for the different assays in order to obtain information under standardized conditions after an accident. The experiments are now continued using different conditions of partial body exposure.

Early after irradiation different systemic reactions take place among which changes in gastro intestinal motivity and, later after higher doses, shock play an important role. We have studied the role of the angiotensin-renin-aldosterone system in the pathogenesis of this shock. Even before a general fall in blood pressure takes place, aldosteron in plasma andurine and renin in plasma increase significantly. Changes were also observed in kidney blood flow and glomerular filtration as well as in the response of blood pressure to catecholamines.

The studies on late biochemical effects of irradiation on the brain have now continued for a period of 18 month after a dose of 2.2 kR. Changes in lysosomal enzymes DNA were observed during the first month after exposure and, later on, an increase in serotonin. Experiments now under way using a higher dose of radiation (4.4 kR) reveal more important changes in biogenic amines already during the early period after exposure.

Biochemical studies in irradiated lung show an increase in lysosomal enzymes as well as changes in fibrolytic activity and DNA. Later, collagen increases as fibrosis develops. The first series of experiments using a dose of 3 kR has been terminated and is now extended to other doses.

G.B. GERBER

Die Erholungsfähigkeit des menschlichen Organismus nach einer Strahlenbelastung. Grundlagen und Empfehlungen.

- Berichte der Schutzkommission am Deutschen Innenministerium(in press)

G.B. GERBER, C. WATTERS

Untersuchungen zum Meçhanismus des gastrointestinalen Syndrom

- Berichte der Schutzkommission am Deutschen Innenministerium. (in press)

G.B. GERBER

Late Biochemical Effects in locally Irradiated Rat Brain

- 5th Internation Congress of Radiation Research, July 14-20, 1974, Seattle, Wash,,U.S.A.

G.B. GERBER

Les indicateurs biochimiques

- Rayonnements Ionisants (in press)

D. KOCMIERSKA-GRODZKA, G.B. GERBER

Lysosomal enzymes in organs of irradiated rats.

- Strahlentherapie 147, 3,(1974) 271-277.

D. KOCMIERSKA-GRODZKA, G.B. GERBER and J.P. DECOCK

Sialic acid and neuraminidase after whole body irradiation of rats

- Acta Radiologica, 13, 1, (1974), 57-64.

M.B. YATVIN, G.B. GERBER and J. DEROO

Effect of age and strain on uptake of α-amino-isobutyrate

- Arch. Intern. Phys. et de Biochimie, 1974, 82, 251-257.

G.B. GERBER, J.P. DECOCK

Analytical methods for Biochemical Indicators of radiation damage.

- Acta Radiologica (in press).

Résultats du projet n° 2

Chef du projet et collaborateurs scientifiques :
R. GOUTIER, W. BAEYENS

Titre du projet : Effects of X-irradiation and radiomimetic substances on the synthesis of ribosomal and messenger RNA's and on the structure and formation of polyribosomes.

In our previous work, we demonstrated differential effects of whole body irradiation on messenger- and ribosomal type RNA's in normal and regenerating rat liver. The technique used in these experiments necessitates the fixation of ribonucleoproteins before their analysis on CsCl gradients. The fixation-step excludes the dissociation of the ribonucleoproteins, necessary for the detection of poly-A stretches that are typical for messenger-type RNA. A new chemical, metrizamide, has been claimed to be a good substituent for CsCl with the advantage of eliminating the formaldehyde-fixation step.
Our results obtained with this chemical, however indicated an unsatisfactory resolution between the two main ribonucleoprotein components present in polyribosomes. The interpretation of the gradient patterns is also seriously hampered by the intrinsic U.V. absorption of metrizamide.
Poly-U substituted Sepharose could be the material of choice for the separation of messenger-(poly-A containing) and ribosomal-type RNA's even from unfixed ribonucleoprotein preparations. This approach will be investigated.

The effects of the combined administration of X-irradiation, thyroid hormones and adrenocorticotropic hormone have been studied in collaboration with Dr M. Lemaire, from the Department of Radiotherapy, University of Liège.

The main conclusions of this work are the following :

1. When given separately, total body X-irradiation, triiodothyronine (T_3) or ACTH produce a stimulation of the liver protein synthesis *in vivo* and *in vitro*.
2. This effect is significantly enhanced in thyroidectomized animals.
3. The stimulation observed by giving X-irradiation and T_3 to the same animals is lower than the stimulation obtained by administrating each treatment separately, indicating a mutual inhibition. This inhibition is tentatively postulated to be mediated by adrenal hormones, released a short time after irradiation during the stress reaction, and inducing the synthesis of some genetic repressor molecules.

We investigated the RNA polymerase activity of nuclear acidic proteins isolated and purified from calf thymus by Dr R. Hacha and Prof. E. Frédéricq (Laboratory of Physical Chemistry, University of Liège. Out of eleven fractions, two only displayed significant enzyme activity. Research on the role of nuclear and cytoplasmic acidic proteins in the radiation syndrome will be further developed in our laboratory. As a first step, we started a study on the influence of cytoplasmic protein factors on the transport *in vitro* of ribonucleoproteins from the nucleus to the cytoplasm. The ribonucleoproteins are prelabelled *in vivo* by injection of ^{14}C-orotate before or after irradiation.

A preliminary study of the experimental conditions has already been achieved by developing suitable methods for the preparation of clean, intact nuclei, the partial purification of the cytoplasmic protein fractions and the optimal incubation conditions. Further attempts to purify and characterize the cytoplasmic factors will be necessary to increase the sensitivity and specificity of the system.

When comparing the transport from nuclei derived from irradiated animals, to the transport from control nuclei, the differences observed are small and depend on the irradiation conditions. A systematic kinetic study is being performed, using one single dose of total body X-irradiation, at different intervals before sacrifice, the nuclei being labelled with various pulses of ^{14}C-orotate.

Résultats du projet n° 3

Chef du Projet et collaborateurs scientifiques :
J.R. MAISIN, G. MATTELIN, M. LAMBIET-COLLIER, C. BIESEMANS-VANGENECHTEN

Titre du projet : Influence of chemical radioprotectors on the long term effects of ionizing radiation.

Our experiments on the effect of sulphydryl radioprotectors given separately or in mixture (2-β-aminoethylisothiouronium-Br-HBr + serotonin + glutathion + mercaptoethylamine + cysteine) on the life span and the causes of death of male BALB/c and C57Bl mice whole body irradiated with single or multiple doses of X-irradiation were continued. BALB/c mice were exposed to 100 or 175 R and C57Bl mice to 350 or 650 R of X-rays, autopsied at death and the tissues analysed with an optic microscope. The results on a total of 1200 mice are summarized briefly :

BALB/c mice

The median survival time of mice irradiated with 100 and 175 R is lower than the median survival time of non irradiated ones (Fig. 1) (for 175 R, $P < 0.01$). The median survival time of the protected irradiated mice is between that of the two other groups but is not significantly different from both (Fig. 1). After small doses of X-irradiation, protection against life shortening can therefore not be ascertained with statistical certainty as has been shown also earlier for acute effects.

Causes of death. The incidence of myeloid leukemias in the non protected mice irradiated with 100 and 175 R is significantly higher than in the control ($P < 0.05$) and in the irradiated (100 R) protected ones ($P < 0.05$). The percentage of lung cancer is more elevated in the mice irradiated with

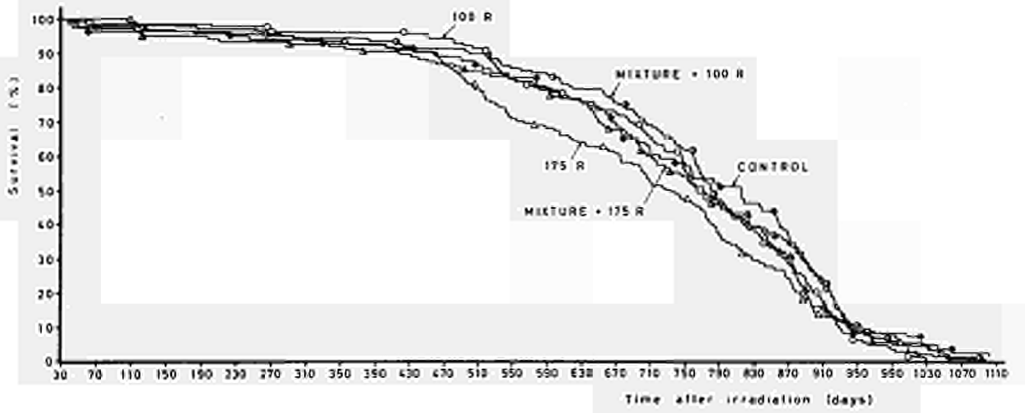

Fig. 1 : Long-term survival of BALB/c mice pretreated or not with a mixture of radioprotectors before X-irradiation with 100 or 175 R.

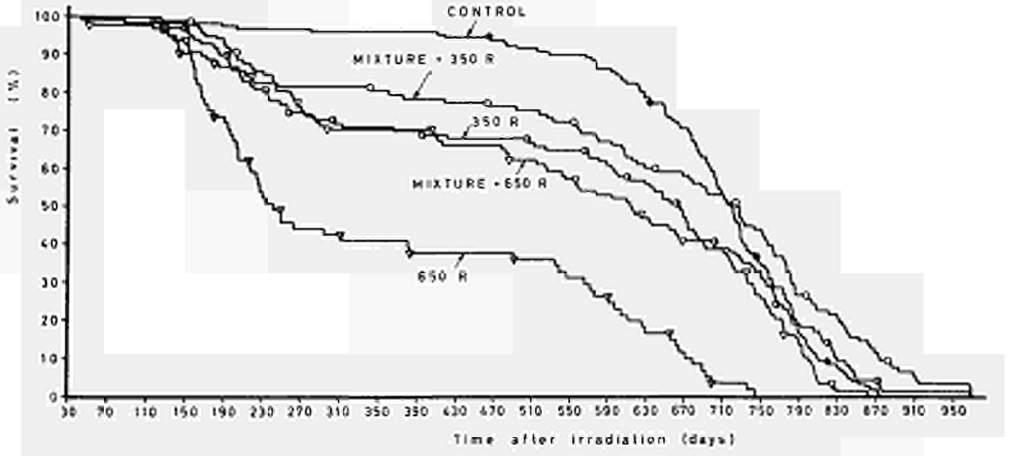

Fig. 2 : Long-term survival of C57Bl mice protected or not with a mixture of radioprotectors before X-irradiation with 350 or 650 R.

100 and 175 R (38.7 % and 34.9 % respectively) than in non irradiated controls (28.9 %) or in protected mice irradiated with 100 R (30.4 %), but these results are not significant as is true also for the incidence of sarcomas.

C57Bl mice

The median survival time of protected mice exposed to doses of 350 or 650 R of X-rays is significantly higher than that of irradiated non protected ones (Fig. 2). The higher survival of the protected mice is due mostly to a lower incidence of thymic lymphoma, The protection by a mixture of radioprotectors against this type of leukemia is, however, not as pronounced than is the case for BALB/c mice. The reason for this difference is now investigated. The incidence of lung carcinoma is significantly higher in mice exposed to a dose of 350 R of X-rays than in non irradiated controls. After an exposure to 350 R of X-rays, the percentage of adenomas or cancers of the liver is significantly higher in the irradiated mice than in the non irradiated ones respectively whether the mice had been protected or not.

J.R. MAISIN
Spontaneous and radiation induced tumors in animals.
- Biomedicine, 20, n° 2, 102-108, 1974.

J.R. MAISIN
Ultrastructure of the vessel wall.
in : Current Topics in Radiation Research Chapter III
Quarterly 10, p. 29-57, 1974.

Résultats du projet n° 4

Chef du projet et collaborateurs scientifiques :
A. LEONARD, G. DECAT, Gh. DEKNUDT

Titre du projet : Study of the chromosome rearrangements induced in male mice by ionizing radiations.

To estimate the genetic hazards of environmental mutagens for human populations it is important to know to what extent the experimental data can be extrapolated from the mouse. In that respect some interesting observations were made recently by Brewen and Preston (1973 ; 1974) who claimed that the frequency of chromosome dicentrics induced in leukocyte cultures by a given dose of ionizing radiations was approximately proportional to the number of chromosome arms of the species. In our experiments the dicentric yield induced by different doses of X-irradiation was studied in the leukocytes of four species having roughly the same number of chromosome arms. The four species used were (Table) Sus scrofa, Ovis aries, Capra hircus and Bos taurus. No consistent difference was observed between S. scrofa and O. aries whatever was the dose at exposure. The number of dicentrics was always higher after exposure of goat leukocytes but, in agreement with the Brewen's results, the best fit for the dicentric data in the three species was to the model $Y = bD + cD^2$. In contrast with the results obtained with S. scrofa, O. aries and C. hircus the exposure of cattle leukocytes to X-irradiation induced a very low yield of dicentrics. Some selective elimination resulting from intensive cell killing could possibly explain this low incidence of dicentrics.

TABLE

YIELOS OF OICENTRIC CHROMOSOMES IN THE FOUR SPECIES

Species	Number of chromosomes	Number of chromosome arms	Oose (R) 100	200	300	400
S. scrofa	38	64	2.00	10.25	36.75	75.00
O. aries	54	60	4.00	9.00	32.00	-
C. hircus	60	60	3.50	17.00	53.00	82.00
B. taurus	60	62	2.50	2.50	2.00	3.75

A. LEONARD, G. DECAT and Gh. DEKNUDT
Chromosome arm number and radiation-induced dicentrics in mammals. (in press).

B. IVANDV and A. LEONARD
Radiosensitivity, to translocation induction, of premeiotic male germ cells of mice of different ages.
Mutation Research, 22, 85-86, 1974.

P.P.W. van BUUL and A. LEONARO
Translocations in mouse spermatogonia after exposure to unequally fractionated doses of X-rays.
Mutation Research, 25, 361-365, 1974.

Gh. DEKNUOT, A. LEONARO and G. DECAT
Influence of blood storage in glass or plactic bottles on the induction of chromosomal abnormalities by X-irradiation.
Toxicology, 3, 87-90, 1975.

Résultats du projet n° 5

Chef du projet et collaborateurs scientifiques :
A. LEONARD, N. GILLIAVOD

Titre du projet : Study of the chromosome rearrangements induced in female germ cells by ionizing radiations.

Our observations on female mice given 0, 25, 50, 100 or 200 R of X-irradiation have been completed and the results compared with the recent data of Searle and Beechey (1974).

RESULTS OF THE OOCYTE EXAMINATION

Treatment (R)	Oocytes examined	Oocytes with anomalies	Chromosome anomalies RIV	CIV	Fragment
0	101	0	-	-	-
25	63	2	2	-	-
50	108	4	3	1	-
100	100	7	1	-	6
200	85	0	-	-	-

Owing to the time-consuming method used, the number of dividing oocytes examined in each group must remain small compared with the numerous spermatocytes that can be analysed in the experiments with male mice. Therefore, the relatively small numbers of dividing oocytes that were studied could possibly explain that, in the present experiments, the percentage of cells with apparent multivalents decreases after doses higher than 50 R whereas it increases up to 400 R in the experiments of Searle and Beechey (1974). However, one cannot exclude the possibility that the C57Bl inbred females are more sensitive than the F_1 (C3H/HeH ♀ x 101/H ♂) females. Because of the comparable radiosensivitivity to cell killing of oocytes in maturing mouse follicles (LD_{100} = 2000 R) and human maturing (LD_{100} = 5000 R) and

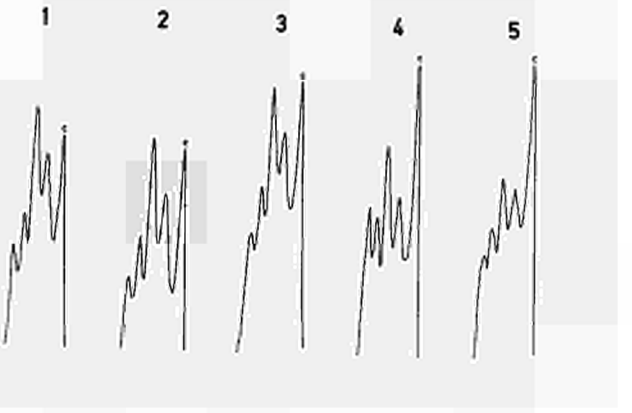

Figure 1
Effect of the duration of trypsinisation on the form of the curve
1. No trypsinisation
2. 5" of trypsinisation
3. 10" of trypsinisation
4. 20" of trypsinisation
5. 30" of trypsinisation

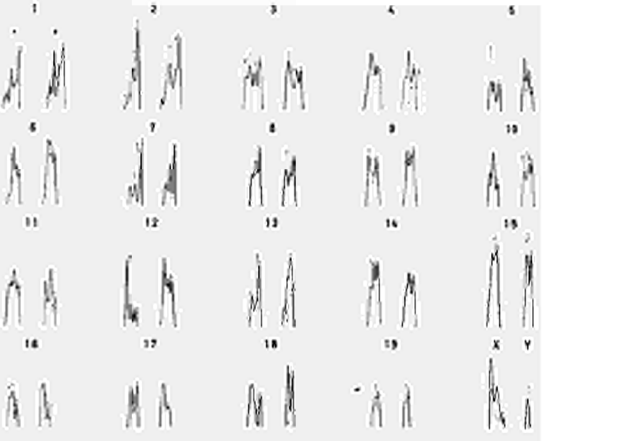

Figure 2
Identification curves of the mouse chromosomes

1, 2, 3, etc.....	:	chromosome pairs
A - B	:	homologous chromosome of each pair
———	:	chromatid one
- - - - -	:	chromatid two
c	:	centromere

primordial (LD_{50} = 2000 R) follicles, the observations on mouse oocytes may permit an estimate of the sensitivity of human oocytes to induction of chromosome rearrangements. If this were so, induction of chromosome rearrangement in human female germ cells and transmission of such translocations to the F_1 offspring represent a potential risk.

N. GILLIAVOD and A. LEONARD
Dose-relationship for translocations induced by X-irradiation in mouse oocytes.
Mutation Research, 25 (1974), 425-426.

Résultats du projet n° 6

Chef du projet et collaborateurs scientifiques :
A. LEONARD, G. DECAT

Titre du projet : Study of radioinduced chromosome aberrations by banding pattern techniques.

A densitometric analysis has been performed on mouse chromosomes from *in vivo* preparations stained with Giemsa after trypsinisation according to the method described in the previous EURATOM report. The photographic negatives or positives are examined by means of a microphotometer consisting of a statif with carrier scanning in XY directions and a photomultiplier with an analog output to a syncronized Y - t , X - Y, or Y - t recorder. The width of the slit used was 0.1 mm for the negatives and 0.25 mm for the positives and the wave length was 560 nm. The characteristic curves obtained (Figure) by analysing the same chromosome after different durations of trypsinisation (1 = 0" ; 2 = 5" ; 3 = 10" ; 4 = 20" ; 5 = 30") suggest that a trypsinisation step is not always necessary to get an analysable banding pattern. No loss of information occurs when trypsinisation time is increased from 0 to 30", the number and position of the different peaks remaining similar. In general the best results are obtained when the chromatids are long and tightly coiled together. Identification of the different chromosomes has been made according to the conclusions of the Committee on Standardized Genetic Nomenclature for Mice.
The identification curves obtained (Figure) for each chromosome and each chromatid were, in general, very characteristic and reproducible in each pair. It must be pointed out, however, that the correspondence between the numbers used in this figure and these recommended by the Committee cannot be considered as absolute.

A. LEONARD and G. DECAT
Identification of the mouse chromosomes by microdensitometry.
(in press).

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München
Institut für Biologie
Abteilung für Strahlenbiologie und Biophysik
Abteilung für Allgemeine und Experimentelle Pathologie

Nr. des Vertrages: 090 - 72 - 1 BIAD

Leiter der Forschungsgruppe: Prof.Dr.O.Hug
Prof.Dr.W. Gössner

Allgemeines Thema des Vertrages:

Pathogenesis of somatic radiation damage

Allgemeine Darstellung der durchgeführten Arbeiten:

The research work 1974 performed in this contract was continued in general along the line proposed in the original research plan and is concerned with the continuation of the previous work outlined in the EURATOM reports 1971, 1972, 1973.

In 1974 the activities are concerned with the following areas of research

- metabolic and dosimetric studies with short-lived β-emitting bone seekers (Y-90, Lu-177, Np-239)
- Th-227 inhalation experiments in rats (in collaboration with CEA, Fontenay-aux-Roses, Dr.Lafuma)
- tumor induction by incorporation of Th-227 in two strains of mice (NMRI and (C3Hx101)F_1) with different spontaneous incidence of leukemia and age related osteoporosis.
- continuation of the Ra-224 experiments: bone tumor induction after dose protraction over 9 months, after bone fracture and in growing and adult mice
- evaluation of long-term experiments with Ce-141 in mice with special regard to soft tissue lesions and tumors
- electron microscopic studies of Ra-224 and Th-227 induced osteosarcoma and search for virus particles in cell culture lines from Ra-224 induced osteosarcoma

- radiation sensitivity of salivary glands of mice after different treatment (isoproterenol, atropin, pilocarpin) prior to X-irradiation
- isoproterenol-stimulated DNA synthesis in the urinary bladder epithelium of the rat
- continuation of epidemiological studies on late effects after medical application of Ra-224 in children and adults

Project 1

W.Gössner, O.Hug, A.Luz, W.A. Müller

Late effects after incorporation of bone-seeking radionuclides

1.Metabolic and dosimetric studies with short-lived α- and β-emitting bone-seekers

The distribution studies with Y-90 (half-life 2.6 days) were completed. Using this radionuclide carrier-free provided a high skeletal retention, but the excessively high β-energy of 2.2. MeV max. causes some kind of a whole-body irradiation for small animals such as mice. Therefore as alternatives β-radionuclides were tested from which could be expected more local skeletal effects as Lu-177 (half-life 6.7 days β-energy max. 490 keV) and Np-239 (half-life 2.5 days β-energy max. 450 keV). Because of its γ-components and its more convenient half-life Lu-177 seems to be preferable for a chronic experiment. Pilot distribution studies showed satisfactory high Lu-177 retention in the skeleton. Detailed distribution studies including dosimetric calculations are underway with Lu-177. In case of Np-239 a higher γ-irradiation (whole-body burden!) has to be taken into account. The skeletal retention is comparable with that of Lu-177, but the shorter half-life necessitates much higher dose rates (for skeleton and whole-body) in the case of Np-239 compared with Lu-177. The liver retention values are lower at least one order of magnitude in case of Lu-177 compared with our first β-experiment using Ce-141.

The method of α-track etching using special solid-state foils is in test. This method should enable us to discriminate different α-energies in a histological slide. This is a very important problem in all our Th-227 studies where thus the different metabolic pathways of Th-227 itself and its daughter Ra-223 could be traced.

Within the EBONY-project of EULEP[+] the first intercomparison retention has been completed. For 1975 a new experiment was decided using Ra-226 and Pu-239 for "Interstrain-Interspecies"

retention studies.

In collaboration with CEA, Fontenay-aux-Roses (Lafuma,EURATOM Contract Nr. 100 - 72 - 1 BIAF) the results of a Th-227- inhalation- experiment were evaluated. Th-227 was inhaled by rats from solutions in nitrate form. Organ doses were calculated after whole-body measurements and measuring of activity concentrations in the organs over a longer incorporation period. An initial deposition of 100 nCi Th-227 in the lung resulted in mean total doses of 150 rad in the lung and 36 rad in bone. The data for kidney and liver were 2 rad and 0.1 rad respectively. For long term experiments two doses were applied to two groups of animals with mean doses of 900 rad and 300 rad in the lung. A competition between lung and bone tumor induction is to be expected.

2. Tumor induction

Incorporation of 5 µCi Th-227 /kg(corresp. to 1000 rads mean skeletal dose) in (C3Hx101)F_1-mice resulted in 60% osteosarcomas in females. This is quite the same result as in the NMRI strain. The result of this inter-strain comparison study seems to be of special interest because female (C3Hx101)F_1-mice in contrast to the NMRI mice have a very low leukemia incidence and develop much more age-related osteoporosis than NMRI mice. The osteosarcoma incidence in (C3Hx101)F_1-males was lower (30%) which is in agreement with the general experience in mice.

Protraction of 36 µCi Ra-224/kg (corresp. to 1080 rads) over 9 months resulted in an osteosarcoma risk of more than 90% during 18 months. After protraction of 72 µCi Ra-224/kg (corresp. to 2000rads) the osteosarcoma risk was more than 80%.

Fracture of both tibiae 16 months after incorporation of 25 µCi Ra-224/kg did not increase the bone tumor risk as compared with the controls after Ra-224 alone.

Since in an experiment with 25 µCi Ra-224/kg the osteosarcoma

risk of 1 month (growing) and 5 months old NMRI female mice was not significantly different we started a new experiment with 5 µCi Th-227/kg with growing and adult mice (expected tumor risk about 60%). In addition to single injection the same total amount of Th-227 was fractionated over 6 months with mice of both age-groups.

As mentioned in the report 1972 the long-term experiment with Ce-141 in mice is difficult to evaluate since in the higher dose range beyond 100 µCi/kg the portion of activity deposited in the bone was decreasing and a large portion of the activity was concentrated in the liver and at the site of injection. With intravenous injection we could not avoid these difficulties since the animals died immediately after injection.

In the long-term experiment with amounts between 170 and 8 700 µCi/kg, from which the lowest group received a mean skeletal dose of 100 rad whilst in the higher dose groups exact data are lacking, we did not observe an increased osteosarcoma risk.

In the highest dose groups occurred degenerative lesions of the liver, chronic fibroblastic lesions (irradiation fibromatosis) of the surfaces of the abdominal organs and the abdominal wall. In some cases soft tissue sarcomas at these sites have been observed. Pilot experiments with intraperitoneal injection of the same chemical amount of inactive Cerium corresponding to the two highest dose groups did not show these distinct lesions of liver and soft tissues during 12 months.

+see EULEP-Report

Project 2

Histogenesis, classification and nomenclature of radiation-induced tumors.

W.Gössner, A.Luz

In May 4-5 and September 23-24, 1974 the 5th and 6th workshop of the EULEP-committee on pathology standardization have been organized.

The main topic of the 5th workshop was "Neoplastic and non neoplastic lesions of the thyroid". Invited speaker was Dr.G. Walinder (Research Institute of the Swedish National Defence).

The main topic of the 6th workshop was "Neoplastic and non neoplastic lesions of the lung". Invited speaker was Dr. H.L. Stewart (Registry of Experimental Cancers, National Cancer Institute, Bethesda /USA).

For further details see EULEP-report.

Project 3

Pathogenesis of early and late effects after internal and external irradiation.

V.Erfle, W.Gössner, A-Luz, K.-H. Marquardt, W.A. Winter

1. Electron microscopic studies on the ultrastructure of radiation-induced bone tumors and search for virus particles.

Electron microscopic studies of Ra-224 and Th-227 induced osteosarcomas of mice (total 16 cases) revealed a rather high degree of cellular differentiation which was not expected from the light microscopic picture. In addition to osteogenic cells abnormous reticulum cells have been observed. Undifferentiated tumor cells showed hypertrophia of the Golgi complex.

Electron microscopic search for virus particles was extended to cell culture lines established from a Ra-224 induced osteosarcoma of a (C3Hx101)F_1 hybrid mouse. The cell cultures contained abundant immature and mature extracellular type C particles. Budding of virus particles from cell membranes was frequently seen (Fig. 1). Additionally, both immature and mature particles occurred intracellularly in membrane-bound vacuoles resembling phagolysosomes. Budding, immature and mature type C particles were also detected in undifferentiated sarcomas of (C3Hx101)F_1 mice produced by intramuscular inoculation of cultured cells in newborn animals.

There seems to be no relation between the intracisternal type A particles, observed in primary Ra-224 induced osteosarcomas (see report 1973), and the type C particles occurring in cell lines and cell line-derived undifferentiated sarcomas.

Several cell culture lines were established from primary Ra-224 induced osteosarcomas and a transplantable Ra-224 induced osteosarcoma. From these cells injected in newborn mice undifferentiated sarcomas have been obtained.

The production of viruses of the oncorna virus group was tested by incorporation of H-3-uridine followed by isopycnic centrifugation of the cell culture supernatant in sucrose, testing concentrated supernatant for RNA dependant DNA polymerase and by infection of mouse embryo cells and of newborn mice with undiluted supernatants. After isopycnic centrifugation a radioactive peak was detected in the density range 1.16-1.18 (Fig.2). Extraction of RNA in the peak fraction revealed a 70 S RNA. Concentrated supernatants showed RNA dependant DNA polymerase activity which was RNase sensitive. No transformations or tumors could be detected in mouse embryo fibroblasts or newborn mice. It is assumed that the osteosarcoma cells contain a RNA sarcoma virus genome which is expressed as a defective particle.

2. Radiation sensitivity of stimulated cell cycle

The radiosensitivity of salivary glands of mice was studied in relation to the amount of secretory material which was modified by pilocarpin, atropin and isoproterenol administrated 1 hour prior to x-irradiation (dose groups 500, 1000, 2000, 3000, 5000 rads as a single dose and the same doses divided into 10 doses over 10 days). The animals were killed 6 months after irradiation.
In the parotid gland high doses caused a marked atrophy of the acini with different histological changes in the less-effected lobuli. In the submandibular gland the most prominent feature was the progressive disappearance of acini with increasing radiation doses. The secretory tubuli were not affected.

In the course of our studies on the isoproterenol-effect on different organs and tissues could be shown, that a single injection of isoprcterenol induced extremely high DNA-synthesis in the rat urinary bladder epithelium (Fig.3).

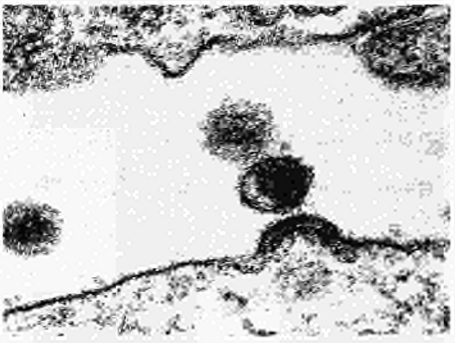

Fig. 1 Three extracellular mature type C particles and one budding particle of a murine osteosarcoma cell line X 160.000.

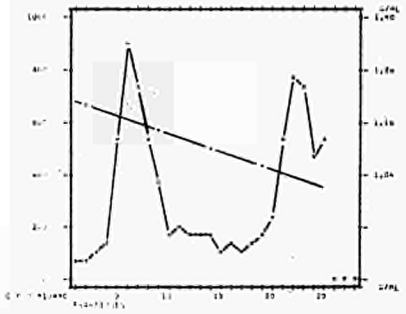

Fig. 2 Isopycnic centrifugation in Sucrose (15-60%) of cell culture supernatant from H-3-uridine treated cells of a Rh-224 induced murine osteosarcoma.
Calculation of data and plotting by EDP.

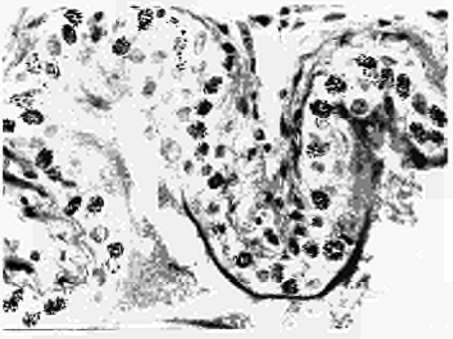

Fig. 3 Autoradiograph of the rat urinary bladder epithelium 24 hours after injection of 60 mg/kg isoproterenol and one hour after H-3-TdR showing a very high number of labelled nuclei.

Project 4

Studies on late effects induced by Ra-224 in children and adults

H.Spiess, A.Gerspach, J. Murken, W.Wiederholt

The results are summarized in table 1.

From the data presently available there seems to be in addition to the development of malignant and benign bone tumors in juvenile and adult patients an increase of liver and kidney diseases and cataract.
A reinvestigation of these lesions has been started.

Table 1 Reinvestigation of Ra-224 treated patients

December 1974

Total number: 1809

not found:241, physicans report without name:654, investigation refused: 16, exactly controlled: 898

Age at beginning of Ra-224 treatment	1-20 years	after 21 years	total number
reply received	218	681	899
report by physician or questionaire	49	325	374
personally explored or at the hospital observed	107	108	215
deceased	67	257	324
causes of death			
main disease	11	41	52
not in relation to main disease or late effects	11	105	116
unknown	4	22	26
malignant bone tumors, osteosarcoma	29 (+3L)	16 (+2L)	45 (+5L)
chondrosarcoma	4	0	4
other malignant tumors (carcinoma)	3 (+2L)	26 (+9L)	29 (+11L)
diseases of the liver (mostly cirrhosis)	1	13	14
diseases of the kidney	3	29	32
panmyelophthisis, anaemia	1	2	3
leukemia	0	3	3
other late effects			
benign bone tumors osteochondroma solitary	7	0	7+
multiple	20	0	20
osteochondroblastoma	1	0	1
fibroma	1	0	1
early broken teeth	19	8	27

delay of growth	50	0	50++
cataract	8	15	23

L = living patients

\+ = of 84 x-ray controls of skeleton

++ = of 99 x-ray controls of skeleton

Project 5

Epidemiological study on late effects after medical application of Ra-224 in ankylosing spondylitis patients

O.Hug, F. Schales

From 1948 up to now about 2000 patients suffering from ankylosing spondylitis have been treated in several German university hospitals by Ra-224 injections.
In 1971 in addition to project 4 a study on possible radiation effects in this group was started. The aim of this study is to calculate the average skeletal radiation doses received by these patients and to correlate them with late effects which may be observed by clinical reinvestigations. The collaboration with 3 hospitals already under contract (Frankfurt, Münster, Hannover) has been continued. The data from other patients from hospitals in Berlin and Kiel are now available.

The causes of death of 141 patients out of 230 deceased patients were checked in the mean time. These patients were treated with relatively low activities. Within this group two cases with diseases of the hemopoietic system were found. The correlation between the internal irradiation and any of the causes of death is doubtful.

This work is done in close contact with project 4 (Prof. Spiess) and using the experiences of the EURATOM thorotrast groups.

List of publications and reports

Erfle,V.F., A.Luz, I.-D. Adler and K.-H. Marquardt
Attempts to recognize an oncogenic virus in Ra-224 induced murine osteosarcoma
5th Intern.Congr. of Radiation Research, Seattle,USA
14.-20.Juli 1974,
Rad.Res.59(1974) 90

Erfle,V.F.
Attempt to recognize oncogenic viruses in radiation induced osteosarcomas in mice
Special seminar, Salk Institute, San Diego/USA
26.Juli 1974

Erfle,V.F.
Die Rolle von RNS-Tumorviren in der Onkogenese und ihr Nachweis bei radionuklid-induzierten Osteosarkomen der Maus
4. Arbeitstagung für Genetik der GSF, Göttingen
8.-10.Mai 1974

Gössner,W., A.Luz, W.A. Müller and O.Hug
Bone tumor risk in mice after single and repeated injection of Ra-224
5th Intern. Congr. of Radiation Research, Seattle, USA
14.-20.Juli 1974
Rad.Res. 59(1974)55

Gössner,W., O.Hug, A.Luz and W.A. Müller
Present status of long-term experimental studies with short-lived α-emitters in mice
Part II: Pathology of Ra-224 and Th-227 induced tumors
Intern. Symp. on "Biological Effects of injected Ra-224 and Thorotrast", Alta, Utah (USA), 21.-23. Juli 1974

Gössner,W.
Experimental induction of bone tumors by short-lived bone-seeking radionuclides
VI. Intern. Symp. "Malignant Bone Tumors", Düsseldorf
17.-18. Oktober 1974

Hautmann,R.
Strahlenwirkung auf die Zellproliferation in der Rattenniere nach unilateraler Nephrektomie und - oder Bleiacetat
Diss.Techn.Univ.München (1974)

Hug,O. and F. Schales
Brief history of Ra-224 usage in radiotherapy and radiobiology
Intern. Symp. on "Biological Effects of injected Ra-224 and Thorotrast", Alta, Utah (USA), 21.-23. Juli 1974

Luz,A. and W.Gössner
Osteosarcomas and age-dependent skeletal changes in mice after incorporation of short-lived α-emitters (Ra-224, Th-227)
10th Intern. Congr., Intern. Academy of Pathology
Hamburg, 16.-21. September 1974

Marquardt,K.-H., A.Luz, W.Gössner and V. Erfle
Virus particles in Ra-224 induced murine osteosarcoma
5th Intern. Congr. of Radiation Research, Seattle, USA
14.-20.Juli 1974
Rad.Res. 59 (1974) 90
Marquardt,K.-H.
Virus-like particles in Ra-224 induced murine osteosarcomas
Beitr. Path. 152 (1974) 116-126

Marquardt,K.-H.,A.Luz und W.Gössner
Zur Ultrastruktur des strahleninduzierten Osteosarkomas der Maus
Verh.dtsch.Ges.Path. 58 (1974) 434-437

Müller,W.A.,A.Luz, W.Gössner and O.Hug
Present status of long-term experimental studies with short-lived α-emitters
Part I: The role of dose and dose rate for bone tumor risk
Intern.Symp. on "Biological Effects of injected Ra-224 and Thorotrast", Alta, Utah (USA), 21.-23. Juli 1974

Müller,W.A., W.Gössner, O.Hug, U. Linzner and A. Luz
Dosimetric aspects after repeated injections of short-lived α-emitters in mice with regard to the risk of late effects
5th Intern.Congr. of Radiation Research, Seattle, USA
14.-20.Juli 1974
Rad.Res. 59(1974) 55

Müller,W.A. und W.H. Müller
Enhanced Ra-224/Pb-212 excretion provoked by cryptating agents in rats
Naturwissenschaften 61 (1974) 455

Pömsl, H.
Frühschäden an Tibia und Wirbel der Maus nach Inkorporation von Th-227 und Ra-224
Diss.Techn.Univ.München (1974)

Spiess, H.
Knochentumoren nach Radionuklid-Applikation
Dtsch.Krebskongress, München 15.2.1974

Spiess, H.
Soft tissue effect following Ra-224 injection into humans
Intern. Symp. on "Biological Effects of injected Ra-224 and Thorotrast", Alta, Utah (USA), 21.-23.Juli 1974

Winter,W.A.
Isoproterenol-induced DNA synthesis in dorsal prostate gland tubules of mice
Beitr.Path. 153 (1974) 73-79

Winter,W.A.
Induction of DNA synthesis by isoproterenol in the rat urinary bladder epithelium
Histochemistry 41 (1974) 141-143

Contractual Partner of the Commission:
Prof.Dr.K.E.Scheer: Director of the Institut für Nuklearmedizin am Deutschen Krebsforschungszentrum, Heidelberg.

Contract No.: 063-72-1 PST D

Head of the Research Group: Prof.Dr.K.E.Scheer, Director of the Institut für Nuklearmedizin am Deutschen Krebsforschungszentrum, Heidelberg,

Assistant Head of the Research Group: Prof.Dr. W.J.Lorenz, Institut für Nuklearmedizin, Deutsches Krebsforschungszentrum, Heidelberg.

Coordinator: Dr. G. van Kaick, Institut für Nuklearmedizin, Deutsches Krebsforschungszentrum, Heidelberg.

The contracted research program is to be performed at the Institut für Nuklearmedizin am Deutschen Krebsforschungszentrum, Heidelberg, in collaboration with:

Prof.Dr. H. Muth, Director of the Institut für Biophysik der Universität des Saarlandes (Boris-Rajewski-Institut), Homburg,

Prof.Dr. G. Wagner, Director of the Institut für Dokumentation, Information und Statistik am Deutschen Krebsforschungszentrum, Heidelberg, and
Prof.Dr. A. Kaul, Klinikum Steglitz der Freien Universität Berlin, Nuklearmedizinische Abteilung.

General Topic of the Contract:

Research Project "Thorotrast" - Investigations to Evaluate the Long Term Effects Caused by Artificial Radiation in Man (Thorotrast-Patients).

General description of the performed work:

The goal of the research project was set down after mutual agreement had been achieved in the coordinating committee attended by representatives of the Bundesministerium für Forschung und Technologie, the Deutsches Krebsforschungszentrum and the Institut für Biophysik der Universität des Saarlandes, Homburg.

The research project Thorotrast, supported by the Bundesministerium für Forschung und Technologie and EURATOM includes:

1. Biophysical and clinical examinations of Thorotrast carriers and of patients belonging into a control group.
2. Discovering the fate of Thorotrast carriers and patients of the control group who have already died.
3. Use of radiological, serological and immunological diagnostic methods for the discovery of Thorotrast induced neoplasias.
4. Follow up examinations of patients of the Thorotrast group and the control group and investigate the cause of the death of patients having died since the last examination.
5. Statistical analysis of the obtained results.
6. Determination of the chromosome aberration rate in Thorotrast carriers and non carriers, and the dependence of this to the radiation dose.
7. Experimental examinations to analyse the foreign body irritation and study the results of the radiation eminating from the thorium dioxide agglomerates.

Results of the Project No. 1:

Head of the Project: Prof. Dr. K.E. Scheer (Contractual Partner), Prof. Dr. W.J. Lorenz (Assistant Head of the Project), Dr. G. van Kaick (Coordinator)

Scientific Collaborators of the Institut für Nuklearmedizin:

Dr. R. Bader, Dr. D. Lorenz, H. Lührs (since 1.6.74), J. Kilian (since 1.5.74), Dr. W. Knapp (since 1.12.73), Dr. P. Schmidlin.

Statistical evaluation: Prof.Dr.G.Wagner, Prof.Dr.H.Immich, Dr. H. Wesch.

Project Title:

a) Search for Thorotrast Patients and for Patients of the Control Group.
b) Clinical and Biophysical Examinations of Thorotrast Carriers and Control Patients.
c) Investigating the Final Fate of Deceased Thorotrast Carriers as well as of Patients in the Control Group.
d) Follow up Studies.

Title a): The search for Thorotrast carriers and patients of the control group was continued in 7 hospitals in the BRD. The former addresses of 850 patients were registered out of the old hospital records. The search for their present address is going on. Patients, who are still alive, were invited for examination. Some Thorotrast patients were announced to us by family or hospital doctors. In most cases the addresses were already known to us. This fact may permit the calculation that the most Thorotrast carriers - as far as possible - were registered by our team.

Title b): In 1974 we examined 268 out-patients from all parts of Western Germany and adjacent countries in Heidelberg. 154 persons came for the first time; 114 patients belonged to the follow-up-study. The examinations were carried out according to the previous criteria and under identical conditions for both groups of patients: clinical examination, x-ray pictures, measurements with the whole body counter,

laboratory findings, immunological tests, sonography, ECG and scintigraphy (if necessary).

The evaluation of x-ray pictures was started for those patients who were examined in Heidelberg. The Thorotrast deposits in the RES of 550 Thorotrast carriers, recognized by means of x-ray diagnosis, were classified as to quantity and morphology. We succeeded in finding regularities of the mode of deposits, which are related to the dose of the injected quantity of Thorotrast. Furthermore 160 paravascular Thorotrast deposits were roentgenologically evaluated, and the x-ray findings were compared with the clinically diagnosed late effects.

Title c): The elucidation of causes of death of deceased patients was continued. X-ray pictures and clinical reports as well as results of post mortem examinations were requested. In the group of Thorotrast carriers 2 primary bone sarcomas were discovered, each with a latency period of 33 years and each secured by histological findings. These findings are significant, as Thorotrast induced bone sarcomas by deposits in the RES are one of the most important problems of the Thorotrast study.

Finally the statistical evaluation was started by giving the coded data of deceased patients into the computer.

Title d): 114 patients were re-examined in 1974. In most cases the first examination dated back 3 years. In the control group the number of old-age-diseases as arteriosclerosis, myocardiosis, emphysema of the lungs, arthrosis etc. was increasing. In the Thorotrast group, however, diseases of the liver were dominating. In the past year we diagnosed 4 primary liver tumors and 10 liver cirrhosis or serious hepatopathies. Furthermore we could find 1 case of aplastic anaemia, 1 plasmocytoma and 2 carcinomas of the lungs. Some of the patients with paravascular deposits of Thorotrast showed local inflammations or new paralysis of nerves, which were compressed by the granuloma. The causes of death of 124 examined Thorotrast carriers could be elucidated:

tumors of the liver	46
cirrhosis	17
leukaemias	5
other neoplastic diseases	17
other non-neoplastic diseases	39

The figures demonstrate that more than 50% of the Thorotrast patients probably died from diseases related to Thorotrast.

Publications:

van Kaick, G., T. Oeftering, W. Wenz:
Urologische Spätschäden bei Thorotrastparavasaten im Leistenbereich.
Radiologe, 14, 182-185 (1974)

van Kaick, G., A. Lorenz:
Kombinierte radiologische Diagnostik zur Beurteilung von Lebererkrankungen bei Thorotrastträgern
in Breit, A., K.-H. Kärcher (Ed.): Gemeinsamer Kongreß der Deutschen und der Österreichischen Röntgengesellschaft 1973, pp. 378-379 (Thieme, Stuttgart 1974)

van Kaick, G., B. Wimmer, D. Lorenz:
Thorotrastinduzierte Neoplasien.
Presented at the Deutscher Krebskongreß München. 14.-16.2.1974.

van Kaick, G., H. Wesch, T. Rogalli:
Das Röntgenbild der Oberbauchthorotrastose und seine klinische Bedeutung.
Presented at the Deutscher Röntgenkongreß, Baden-Baden. 25.-27.4.1974

van Kaick, G., H. Muth, D. Lorenz, A. Kaul:
Malignancies in German Thorotrast Patients and Estimated Effective Radiation Dose.
Presented at the International Symposium on Biological Effects of Injected ^{224}Ra (ThX) and Thorotrast. Alta, Utah, USA, 22.-23.7.1974

van Kaick, G., D. Lorenz, W.J. Lorenz, K.E. Scheer:
Epidemiological Study on Late Effects of Thorotrast in West Germany.
Presented at the 11th International Cancer Congress, Florenz. 20.-26.10.1974

Project 2: Working Group "Institut für Biophysik der Universität des Saarlandes, 665 Homburg/Saar"

Head of Project: Prof. Dr. H. Muth

Title a) Clinical an Biophysical Examinations of Thorotrast Patients

Scientific Collaborators: Prof. Dr. H. Muth
Ass. Prof. Dr. W. Kemmer
Prof. Dr. Dr. E. Oberhausen
Dipl. Phys. A. Steinsträßer

Title b) Chromosome Aberrations Caused by Thorotrast

Scientific Collaborators: Ass. Prof. Dr. W. Kemmer
Prof. Dr. H. Muth

Technical Collaborators: H. Becker/U. Borkenhagen

Title c)Radiation and Non Radiation Effects of Thorotrast, in team work with the working groups Prof. Dr. A. Kaul, Berlin and Heidelberg

Scientific Collaborators: Ass. Prof. Dr. W. Kemmer
Prof. Dr. H. Muth
Dipl. Phys. A. Steinsträßer

RESULTS OF PROJECT 2:

Title a): The studies on Thorotrast patients who have been unable to come to clinical examinations to Heidelberg or Homburg were continued in 1974. A team of a physician and a biophysicist examined these persons in their home. In these patients the concentration of Thoron in the exhaled air was measured by a special equipment, transported in a van. The ^{224}Ra-equivalent value calculated by the amount of Thoron is in direct relation to the whole body counting. The X-ray examinations were made by a local radiologist, if it was possible. In most cases we got all examination results, necessary for the questionnaires and the documentation. Up to now we have examined 103 of these persons. Of whom 82 were Thorotrast patients. The investigations and preparations for a new examination

of 5o persons were finished in the winter of 1974, these patients will be visited in March-April 1975.

Title b): Further details on the dose-effect-relationship between the radiation dose and the chromosome aberration rate in Thorotrast patients can only be obtained by animal experiments under statistical conditions. In collaboration with the working group in Berlin, "in vivo" experiments with Chinese Hamsters were started. These animals are very suitable for cytogenetic studies because the blood sampling for chromosome examinations is very easy, the number of chromosomes is low (2n = 22) and the chromosomes are very long, similar to human chromosomes.

Three groups of animals were injected with different amounts of "Thorotrast", prepared by Prof. Kaul's working group in Berlin (see also Part 3 of this report):

Group 1 : Injection of four different amounts of normal Thorotrast

Group 2 : Injection of four different amounts of Thorotrast, enriched with Th-230 (the α-energy-emission rate is higher than in normal Thorotrast by a factor of 5)

Group 3 : Injection of different amounts of Thorotrast, enriched with Th-23o (the α-energy-emission rate is higher than in normal Thorotrast by a factor of 10)

Control Group : The animals were injected with dextrine-solution (20%) for control, because every charge of Thorotrast contains dextrine for stabilisation

In all these experiments described, the number of treated animals was 15 for each amount of Thorotrast or dextrine resp.

The chromosome aberration rate in Chinese Hamsters treated with Thorotrast will be compared with results obtained from animals irradiated with ^{60}Co γ-rays. The γ-irradiation will enable us to find a dose-effect-relationship between the radiation dose and chromosome aberrations as a calibration curve. These results can be compared with the Thorotrast results. The γ-irradiation is going to begin

in April 1975. All animals will be examined cytogenetically and -after death- pathologically.

Title c): Foreign body experiments with Chinese Hamsters using non radioactive "Zirkonotrast" can only be started, if enough animals are available. The breeding of Hamsters is a little difficult but in the spring of 1975 we will have bred enough Hamsters for these experiments.

Publication:

KEMMER, W., F. Tranekjer, and U. Borkenhagen: CHROMOSOME ABERRATIONS AND BIOLOGICAL DOSIMETRY AFTER RADIATION EXPOSURE - Results, Current Examinations, an Future Developments

European Meeting on Biological Radiation Dosimetry, "Chromosome Aberrations as an Indicator of Radiation Dose"

Centro studi e controlli dell' ENPI, Rome, Nov. $12^{th}/13^{th}$ 1974

Results of Project Nr. 032-67 PSTD

Part 3:
Research Group Klinikum Steglitz der Freien Universität Berlin

Head of the Project: Prof. Dr. A. Kaul
Scientific Collaborator: Dr. W. Riedel
Technical Collaborator: B. Müller / G. Witzke
Title of the Project:
Dosimetry, Foreign Body and Radiation Effects of Thorotrast

Part 3a:
Tissue Distribution, Steady State Activity Ratios and Dose Rates in Man following intravascular Injection of Thorotrast

In addition to former investigations on tissue distribution and steady state activity ratios of ^{232}Th and daughters recalculations of absorbed doses were performed for patients with longterm Thorotrast burdens, with special emphasis to bone dosimetry.

Soft tissue doses:
For an average Thorotrast burden of 25 ml, and 30 years to malignancy the mean α-ray dose to the liver is 750 rd. Assuming an average Thorotrast burden of 15 ml in those patients of the German Thorotrast study who had died from leukemia about 30 years after Thorotrast administration the total mean α-ray dose to the bone marrow yielded to be 170 rd. The mean α-ray dose to the various parts of the lungs range from 60 to 620 rd for the condition of a 30 years Thorotrast burden of 25 ml. The corresponding kidney dose proved to be 130 rd. Generally the contribution of β- and γ-rays may be neglected as both radiation types will contribute to the total dose only up to 10 %.

Bone tissue doses:
The average α-ray dose rate to the skeleton proved to be about 120 mrd/yr per ml intravascularly injected Thorotrast. The concept of calculating mean skeletal doses, however, seems to be unsuitable for estimating risk coefficients for radiation carcinogenesis in bone, as cells on bone surfaces are suggested to be those with the

greatest proliferative potential and thus with the greatest susceptibility to tumor formation. Consequently dose rate calculations and bone sarcoma risk estimates for ^{232}Th and daughters were performed separately for the various parts of bone tissue basedupon the ICRP retention functions for whole body, bone surface, and new and old compact and cancellous bone.

The average skeletal dose rates for various parts of bone tissue range from 0,1 to 0,25 rd/yr per ml intravascularly injected Thorotrast. The average total skeleton α-ray dose rate from ^{224}Ra "translocable" to bone surface has proved to be 0,024 rd/yr x ml, corresponding to an α-ray dose of 18 rd for a 30 years period of 25 ml Thorotrast burden. If applied to the SPIESS/MAYS postulate of a linear relationship between bone sarcomas and average skeletal dose of 0,9 - 1,7 % incidence per 100 rd one should expect 1,6 - 3,1 spontaneous primary bone cancers per 1 000 Thorotrast patients. This number is in good agreement with an estimate by ROWLAND et al. who expected 2,4 to 4,6 cases of bone cancer per 1 000 Thorotrast carriers.

Part 3b:
Foreign Body and Radiation Effects of Thorotrast

In order to evaluate foreign body and radiation effect of Thorotrast, animal experiments with rats are provided by application of "normal" and "enriched" Thorotrast. Normal Thorotrast contains ^{232}Th, together with ^{230}Th at a degree of less than 10 % activity. By addition of ^{230}Th to natural thorium, samples of colloidal thorium dioxide of different α-energy-emission-rates may be produced.

Methods and Materials:
As the basis for the production of ^{230}Th enriched Thorotrast, a $^{230}ThO_2$ (Union Carbide Corp., Oak Ridge Tenn.,USA) was used. The ThO_2 was mixed together with $NaHSO_4$ and heated thereby resulting a soluble salt. This salt was then dissolved in a diluted HNO_3 and adjusted to a definite volume. A calculated aliquot of this stock solution was then added to a solution

containing a corresponding concentration of natural $Th(NO_3)_4 \cdot 8H_2O$ thus resulting in a definite activity relation between ^{232}Th and ^{230}Th. Out of this solution thoriumoxalate was precipitated. Thoriumoxalate was further refined to ThO_2 - sol equivalent to commercial Thorotrast using the recently described methods. (W. Riedel, B. Müller, A. Kaul, Non-Radion Effects of Thorotrast and other Colloidal Substances Risø Report No.294, 1973, Proceedings of the Third International Meeting on the Toxicity of Thorortrast.)

Results:

Four different charges of enriched "Thorotrast" were prepared, containing α-energy-emission rates which were by factors of 2, 5, 10 and 50 resp. higher than in "normal Thorotrast". Normal and enriched Thorotrast will be applied to the animals in volumes from 60 to 600 µl per animal. Comparing the frequency of observed long-term effects within the different animal groups the fraction of foreign body and radiation in tumor genesis may be estimated.

Publication:

Kaul, A., H. Noffz: Tissue Dose in Thorotrast Patients. Presented at the International Symposium on Biological Effects of Injected ^{224}Ra (ThX) and Thorotrast. Alta, Utah, USA, 22.-23.7.1974.

Contractant de la Commission:		Commissariat à l'Energie Atomique (France)
N° du contrat	:	100-72-1 BIAF - BT II - 13.202 D 4
Chef des Groupes de Recherche:		Dr. J. LAFUMA
Thème général du contrat	:	Métabolisme et action toxique des radioéléments.

Les travaux réalisés en 1974 développent les études sur l'action toxique des radioéléments. Les études comprennent deux parties:

- action des radioéléments inhalés
- action des émetteurs β (Cerium 144) administrés localement.

D'autres recherches ont été pratiquées sur la décorporation et les mécanismes d'épuration alvéolaire.

COMPOSITION DU COMITE DE GESTION.

Dr. JAMMET Président

M. BRESSON Secrétaire

M. BERTINCHAMPS

Dr. RECHT

M. de SADELEER

M. LAFFAYE

RESULTATS du PROJET n° 1 :

Chef du Projet : Dr. J. LAFUMA

Collaborateurs scientifiques : Dr. J.C. NENOT
Dr. W. SKUPINSKI
Melle M. MORIN

Titre du projet : Action toxique des radioéléments.

I/ RADIOELEMENTS INHALES.

1/ Emetteurs α : Les études se sont poursuivies avec les différents actinides. Les recherches sur l'action toxique ont montré que la durée de la vie était d'autant plus raccourcie que le radioélément était mieux dispersé dans le poumon. On déduit des résultats un facteur d'hétérogénéité qui a les valeurs suivantes en allant du Nitrate de Cm244 (monomerique) à l'Oxyde de Pu239 (particulaire).

Les valeurs sont les suivantes:

Eléments	Facteur H
- Nitrate de Curium 244	1
- Oxyde de Pu238	1.5
- Nitrate de Pu238	2.
- Nitrate d'Am241	2.3
- Oxyde d'Am241	3.
- Nitrate de Pu239	5.
- Oxyde de Pu 239	5.5

Les expériences sur l'induction des cancers pulmonaires par le Pu239 et le Nitrate d'Am241 sont terminées . Elles ont donné les résultats suivants exprimés en "rads équivalents Curium 244" en tenant compte du facteur H. Les résultats sont réservés dans les tableaux 1 et 2.

TABLEAU 1

Americium 241 Nitrate = (10^9 α 37 rads)

"Rads"	N(rats)	K(cancers)	$\frac{k}{N}$ X 100	% min	% max	nano-curies à t_o
170	12	2	16	3	47	130
290	19	3	16	4	38	240
830	20	15	75	52	92	640
1300	17	10	60	35	77	1000

TABLEAU 2

Plutonium Oxyde de Pu239 ($10^9\alpha$ 15 rads

"Rads"	N(rats)	K(cancers)	$\frac{k}{N}$ X 100	% min	% max	nano-curies à t_o
165	14	7	50	23	76	45
200	9	3	33	9	68	50
265	8	5	62,5	25	92	80
340	18	13	72	47	92	95
550	10	6	60	26	87	135
650	16	9	56	32	77	170
1300	33	22	66	48	82	350
		Nitrate de Pu	($10^9\alpha$	16 rads)		
560	18	16	89	70	97	240

II/ CERIUM 144 ADMINISTRE LOCALEMENT:

Des injections intramusculaires d'hydroxyde de Cerium 144 ont été pratiquées avec des activités allant de 2 à 20 Microcuries.

On a observé d'une part un raccourcissement de la vie et, d'autre part, dans les neuf dixièmes des cas,

l'apparition de sarcomes locaux (plus de 50 cas) de types histologiques variés.

Les études ne sont pas achevées mais il ne semble pas qu'il y ait dans les conditions expérimentales, de relations entre l'activité injectée et les effets produits.

Publications.

LAFUMA J. - NENOT J.C. - MORIN M. - MASSE R. - METIVIER H. NOLIBE D. - SKUPINSKI W.
Respiratory carcinogenesis in rats and monkeys after inhalation of radioactives aerosols of actinides and lanthanides in various physico-chemical forms
Congrès on " Experimental Respiratory Carcinogenesis and Bioassays - SEATTLE - 23/26 Juin 1974.

LAFUMA J. - MASSE R. - METIVIER H. - NOLIBE D. - NENOT J.C. MORIN M. - PERRAUT R. - CHAMEAUD J. - SKUPINSKI W.
Etude expérimentale des polluants radioactifs inhalés: I/Données actuelles, II/ Inventaire lésionnel, III/ Validité du modèle animal. Relations dose-effet -
Colloque de Pont-à-Mousson - 18/19 J nvier 1974.

Résultats du projet n° 2.

Chef du projet : Dr. J. LAFUMA

Collaborateurs scientifiques : H. SCHORN
R. BATTI

Titre du projet : Etude de l'épuration alvéolaire et action toxique de certaines molécules chimiques.

Ce projet comporte deux parties:

- 1/ Action des stéroïdes sur l'épuration alvéolaire. Les résultats ont été rassemblés dans un rapport qui a été présenté dans un congrès sur les hormones steroïdiennes.

Publication:

SCHORN H. - LAFUMA J.
Influence of corticosteroïds on the phagocytosis in the Lung.
Livre des Proceedings of Steroïd Congress - 4th Congress on Hormonal steroïds - MEXICO - 2/7/1974 -

- 2/ Action toxique du Methyl mercure.

Une méthode de dosages par chromatographie en phase gazeuse a été mise au point et un certain nombre de mesures pratiquées. Les résultats ont été publiés dans les articles suivants:

BATTI R. - ARNOUX B. - LAMY G. and MASSE R.
Thin Layer and Gas-Chromatographic analysis of Alveolar Lipids in Silicotic Rats
-EUR 5123

BATTI R. - MAGNAVAL R. - LAMY G. - LAFUMA J.
Détermination de faibles teneurs de mercure organique et inorganique par chromatographie en phase gazeuse.
-EUR 5085

BATTI R. - MAGNAVAL R. - LANZOLA E.
Methylmercury in River Sediments
Chemosphere 1975, Vol.4, N° 1

MAGNAVAL R.- BATTI R. - BOUVILLE A.
Evaluation de la charge corporelle en mercure par simulation numérique.

Progrès récents dans l'évaluation des effets de la pollution de l'environnement sur la santé.
PARIS - 24/28 Juin 1974.

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Résultats du projet n° 3.

Chef du projet : W.H. MÜLLER

Titre du prohet : Etudes de décorporation

Les études de décorporation se sont spoursuivies pour divers éléments:

I/ Cs 137 et Bleu de Prusse.

Les résultats ont été rassemblés dans une publication:

MÜLLER W.H. - DUCOUSSO R. - CAUSSE A. - WALTER C.
Long-Treatment of Cesium 137 contamination with colloïdal
- Strahlentherapie -

II/ Etude des cryptates.

1/ Une étude technique a été pratiquée pour la détermination par gravimètre de quelques cryptateurs:

MÜLLER W.H. - BEAUMATIN J.
A gravimetric method for the determination of some cryptating agents.

2/ Des expériences ont été pratiquées en collaboration avec le Dr. Walter MÜLLER qui travaille à l'"Institut für Biologie der Geselschaft für Strahlen- und Umweltforschung m.b.H., NEUHERBERG " pour étudier la décorporation du plomb et du Radium.

Les résultats montrant l'efficacité de la méthode ont été rassemblés dans la publication suivante:

MÜLLER W.H. - MÜLLER W.A.
Enhanced $^{224}Ra/^{212}Pb$ excretion provoked by Cryptating Agents in rats.
- die NATURWISSENSCHAFTEN - 61. 1974, 455.

III/ Synthèse de chélateurs.

W.H. MÜLLER
Synthese der Äthylester von NTA, ÄDTA, DTPA und Pyridin-2.6-Dicarbonsäure mit Hilfe von Pyrokohlensäurediäthylester
- Archiv der Pharmazie -

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Contractor:	National Radiological Protection Board
Contract No.:	132-74-1 BIO UK
Head of research team(s):	Dr. G. W. Dolphin
General Subject of Contract:	Binding of actinides to mammalian proteins

Information on the binding of actinides to mammalian proteins has been directed towards producing therapeutic methods of enhancing the excretion of plutonium from the rat. Because of the known association of hepatic plutonium with the lysosomes, experiments have been carried out using various drugs which act on the lysosome. None of the pharmological procedures under test lead to an increased plutonium excretion.

Results of Project No.: 1

Head of Project and Scientific Staff: D. S. Popplewell
B. W. Loveless

Title of Project: Binding of actinides to mammalian proteins

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1. Administration of phenobarbitone

It was considered possible that hepatic plutonium might exist in a soluble form, bound to either low molecular weight metabolites or protein molecules within the lysosomes, and thus an increased flow of fluid through the liver might enhance excretion. A group of rats having hepatic plutonium burdens were given phenobarbitone as 0.1% (v/v) solution in their drinking water, a treatment which is known to increase bile excretion. However, rather than promoting the release of plutonium from the liver, the treatment appeared to aid its retention. This effect may result from the proliferation of the endoplasmic reticulum known to be produced by phenobarbitone.

2. Lysosomal labilisation

The administration of large doses of vitamin A has been shown to increase the permeability and fragility of the lysosomal membrane, resulting in the release of contents; no reduction of hepatic plutonium burdens was found in rats to which 800 mg/kg of retinyl acetate was given over a 3-week period.

3. Iron unloading

There is evidence that plutonium may enter the lysosome as a complex with ferritin, which is the principal iron-storage protein of the liver. The procedure of iron unloading, which is known to cause the expulsion of intralysosomal ferritin into the bile, was therefore investigated. However there was no evidence of any stimulation of plutonium excretion during this treatment, and it was concluded that ferritin does not appear to be a major site of intralysosomal plutonium binding.

Publication:

Studies of the removal of intracellular plutonium. B. W. Loveless. National Radiological Protection Board Report Series No. 31, 89-102, 1975.

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

Contract No.: 076-74-1 PSTUK

Head of research team: A. Morgan (Project No. 1)
A.C. Chamberlain (Project No. 2)

General subject of contract: RADIOACTIVE AEROSOLS

Project 1. Uptake and clearance of inhaled vapours

Monodisperse polystyrene particles 2.5, 5.0 and 7.5 μm diameter labelled with technetium-99m (half-life 6 hours) were administered to 10 subjects by inhalation. The aerosols were inhaled by mouth at a constant respiratory rate (10 breaths/minute) and free tidal volume over 2-3 minutes. Total deposition was measured by sampling the inhaled and exhaled aerosol through filters and counting the radioactivity associated with each. Immediately following administration the volunteers washed out their mouths and the number of particles deposited in the mouth and throat was found by radiometric means. Pulmonary deposition was measured by monitoring the chest radioactivity on each subject's centre line and at positions 10 cm to either side for the 24 hours following administration. It was assumed that particles deposited in the tracheobronchial (TB) region were completely cleared by ciliary action within 24 hours and that the fraction of the initial counting rate remaining represented the fraction of particles deposited in the pulmonary (P) region. Unfortunately, there was interference in the chest region from activity in the gastro-intestinal tract. A correction was calculated for the left side of the chest by calibrating the counts from a 'stomach' detector using data obtained from ingestion experiments with 4 of the volunteers.

Project 2. Assessment of internal contamination with americium-241

Study of the behaviour of americium-241 in eight men

Techniques of body radioactivity measurement have been employed to investigate the retention and distribution of americium-241 in eight subjects who were accidentally exposed to airborne contamination. Serial measurements of whole body content have been made using arrays of sodium iodide crystals distributed about the subject to detect the 60 keV gamma radiation, while the redistribution of the inhaled material from its

initial site in the lungs is being followed with individual detectors viewing selected anatomical regions.

Calibration of detectors used for the assessment of americium-241 in lungs

Conventional methods of calibration, employing activity incorporated into phantoms, may not be reliable for the assessment of lung contamination with 60 keV gamma-ray emitters if an accuracy of ± 30 per cent or better is envisaged, unless the phantom is realistic anatomically and account is taken of the potential dependence of response on the subject's physique. An alternative approach is the administration to volunteers of short-lived radioactive aerosols emitting radiation of similar energy. A technique has recently been developed at Harwell for producing lead-203-labelled motor exhaust aerosols, for use in studies of the metabolism of exhaust lead in humans. Lead-203 emits X-radiation at 73 keV, similar in energy to the gamma-rays of americium-241, and also gamma-rays at 279 keV which enable the amount deposited in the subject's lungs to be determined independently using established techniques of whole body counting. Detection of the 73 keV X-rays with counters viewing the chest enables calibration data for americium-241 in lungs to be derived, and data for subjects covering a range of body weights are being accumulated.

Results of Project No. 1

Head of Project and scientific staff: A. Morgan
N. Foord

Title of project: DEPOSITION AND CLEARANCE OF INHALED MONODISPERSE PARTICLES IN THE HUMAN RESPIRATORY TRACT

Of the 10 volunteers, 1 was a smoker and 2 showed considerable chest clearance between 6 and 24 hours. Since the in tial work was to study non-smoking healthy subjects in which TB clearance was complete well within the 24 hour period, these subjects have not been included in the results.

The average total and mouth deposition of particles are given in Table 1.

Table 1

Nominal particle diameter (μm)	Total Deposition		Mouth Deposition	
	Average (%)	Range (%)	Average (%)	Range (%)
2.5	69	57 - 78	4.8	0.8 - 14.9
5.0	86	74 - 97	17.0	1.0 - 52.4
7.5	95	90 - 97	39.8	5.9 - 76.8

Particularly noticeable in these figures is the large subject-to-subject variation observed.

The percentages of initial radioactivity retained in the left and right side of the chest after 24 hours are given in Table 2.

The correction for the contribution from the gut region is included and the final retained activity for the left chest is given.

Table 2

Nominal particle diameter (μm)	Uncorrected chest retention		Stomach correction (%)	Corrected retention Left (%)	Corresponding TB/P deposition ratio
	Left (%)	Right (%)			
2.5	88	85	4	84	0.19
5.0	71	72	6	65	0.54
7.5	45	52	15	30	2.33

It appears that for particles $\leqslant$ 5 μm the contribution from the gut region was small, and the resultant errors would have been small if it had been ignored. However, for larger particles, > 5.0 μm, this was not true and a correction was necessary to obtain a relevant result. Work is continuing to obtain a better correction for activity in the gastro-intestinal tract.

The clearance curves for 7.5 μm diameter particles clearly showed the initial rapid clearance from the TB region followed by the substantially slower clearance from the P region. An exponential function was fitted to the TB clearance from which a half life was calculated. The average half life was 85 min (SD = 46). Several volunteers exhibited an 'induction' period during which there appeared to be either no clearance or a redistribution of particles creating an apparent increase of the activity in the chest region.

The results obtained for the one heavy smoker included in the series showed that his TB/P deposition ratio was significantly different from non-smokers - 0.75, 2.7 and 9.0 for 2.5, 5.0 and 7.5 μm particles respectively. However, there was no significant difference in his initial rapid clearance rate (91 min half life) except that owing to a higher TB deposition this rapid clearance was apparent for 2.5 μm particles whereas for other subjects it was not.

Results of Project No.: 2

Head of Project and scientific staff: D. Newton
Miss F.A. Fry
M.C. Eagle

Title of Project: ASSESSMENT OF INTERNAL CONTAMINATION WITH AMERICIUM-241

Study of the behaviour of americium-241 in man

Subject CR, who inhaled americium-241 in 1971, has been studied in most detail. His whole body content has been measured on eight occasions between days 153 and 1392 after the assumed time of intake. The average result for this period, during which no downward trend could be discerned, was 59 ± 3 nCi (standard deviation). Thus elimination from the body has been negligible, although major changes in distribution have occurred.

The response of detectors viewing Subject CR's lungs has declined suggesting a reduction in the lung burden from 28 nCi on day 153 to 9 nCi on day 1392; the data for days 238 - 1392 are adequately fitted by a single exponential function of time, corresponding to a biological half-life of 884 ± 117 days, but this may be an overestimate because activity present in other organs may have contributed to the response of the counter. There are also indications of an earlier, more rapid phase of lung clearance.

The use of detectors viewing selected anatomical regions has shown that while the liver content has remained approximately constant, the skeletal burden has increased at least by a factor of two between days 324 and 1392. From these observations the distribution of americium-241 within the body can be estimated very approximately, as follows. On day 324, lung ~35%, liver ~50% and bone ~15%; on day 1392, lung ~16%, liver ~50% and bone ~34%.

In six other subjects involved in the same incident, the presence of activity in liver and bone has been established and whole body contents (in the range 8 - 26 nCi) have remained constant over a period of three years since the initial investigations approximately 250 days after exposure. In one subject (ED) a component of half-life 1077 ± 264 days has been observed in the lung clearance; in four others estimates range

from ~500 to > 2000 days, although in each case these are based on the results of three measurements only.

In Subject MB, whose contamination was discovered only two and a half years ago but may have been acquired as early as 1962, the behaviour of americium-241 appears to be quite different. The lung burden of 14 nCi did not decline between June 1972 and September 1973, and there is no evidence of uptake by liver or bone. There is however indirect evidence that the apparent lung burden may include activity which has migrated to the pulmonary lymph nodes.

Calibration of detectors used for the assessment of americium-241 in lungs

Five volunteers, covering the range 58 - 88 kg body weight, have so far inhaled the lead-203 aerosols on two or more occasions. In most subjects the response of the detector in the two positions (viewing the front and back of the thorax) was the same within ± 15 per cent; however in one experiment the heaviest subject (88 kg) gave a 50 per cent higher response from the back. Derived calibration factors for americium-241 (sum of response in two positions) were in the range 56 - 88 counts per minute per nanocurie (approximate energy range 12 - 90 keV). There was no obvious correlation of detection efficiency with physique, although the expected trend towards lower calibration factors in heavier subjects may have been obscured by the effects of other possible variables, such as the pattern of deposition of the aerosol. Efforts will be made to resolve these uncertainties as further data become available.

Commission Associate: ENEL - Ente Nazionale Energia Elettrica

Contract number: 062 - 72 - 6 - PST 1

Head of research team: Prof. Antonio Farulla

General subject of the contract: Effects of prolonged exposure to low levels of ionizing radiation; morphological, cytochemical and cytogenetic researches on circulating lymphocytes of subjects professionally exposed to the hazard of ionizing radiation in a nuclear power station of Enel.

This report concerns the results of the cytogenetic analyses on circulating lymphocytes from workers professionally exposed to ionizing radiation. During 1974 the cytogenetic studies were performed on 46 workers of Enel nuclear power plant and on 12 unexposed persons, of male sex and clinically normal, 25 to 50 years old, wich formed the control group.

The research activity during 1974 includes the continuation of investigations carried out during 1973 on biochemical and histochemical modifications of some metabolic parameters during lymphocyte transformation in culture. Among the metabolic changes, the most important from the point of view of radiation protection are those concerning enzymatic activities and RNA synthesis, in that these changes may represent very sensitive tests of cell damage caused by very small doses of radiation such those absorbed by professionally exposed workers.

Publications.

A. Farulla, G. Naro et alii: Ciclo cellulare in coltura e radiazioni ionizzanti. Securitas 59, n. 5-6, 1974

A. Farulla, G. Naro et alii: Effetti delle radiazioni ionizzanti e di inibitori metabolici sulla sintesi dell'RNA di linfociti in coltura. Securitas 59, n. 5-6, 1974

A. Farulla et alii: Stabilization by sphyngomyelin of calf thymus histone-DNA complexes. IRCS 2, 1232, 1974

G. Naro: Le alterazioni cromosomiche da basse dosi di radiazioni. Seminario Europeo sulla Dosimetria Biologica delle Radiazioni, Montepor zio Catone 12-13 novembre 1974

Results of project n. 1

Head of project and scientific collaborators:
A. Farulla - A. Manzoli - V. Monesi - G. Naro

Project title: Effects of prolonged exposure to low levels of ionizing radiation: morphological, cytochemical and cytogenetic researches on circulating lymphocytes of subjects professionally exposed to the hazard of ionizing radiation in a nuclear power station of ENEL.

Cytogenetic Surveys.

The culture procedures and the materials used have been described earlier. The cultures were harvested after 48 - 52 hours in order to examine the cells at their first mitosis in culture. The examination included the chromosome number and all types of aberration both chromatid and chromosome. The absorbed doses accumulated by the professionally exposed persons ranged, during 1974, from 600 mrem to 4 rem; the total dose accumulated during the entire course of professional activity (8 - 10 years) ranged between 14 and 26 rem. This dose falls therefore within the maximum permissible levels.

46 cultures of blood obtained from professionally exposed people were performed; a total of 3.910 metaphase plates were examined; the number of plates examined was between 70 and 100 in the different cultures.

The frequency of aneuploidy was 5,3% (185 hypo and 22 hyperdiploid cells). 385 cells showed chromatid type aberrations (7,8%). Chromosome type aberrations were never observed.

In 12 cultures performed with blood obtained from the control subjects, 900 mitotic cells were examined (as on average 75 cells per culture). The frequency of aneuploidy was 5% (36 hypo and 9 hyperdiploid cells). 4,8% cells carrying chromatid type aberrations and no cells with chromosome type aberrations were observed.

The results obtained confirm those reported previously: with doses below the maximum permissible doses chromosome type aberrations are absent. This conclusion refers obviously to a total accumulated dose of 26 rem, as that observed in the cases under study.

A useful method for the study of the biological effects of the chronic exposure to small radiation doses may be that of the chromosome banding techniques. This study was recently initiated in a few cases.

Histochemical and metabolic research.

Material and methods. Two types of culture were used: cultures in Petri dishes for the histochemical and autoradiographic experiments and cultures in universal containers for the evaluation of the incorporated radioactivity by liquid scintillation counting.

Cultures in Petri dishes. Suspension of human leukocytes were obtained with the method described earlier. 2×10^6 cells, suspended in 5 ml of TC 199 medium with 15% calf serum were seeded in Petri dishes containing slides and incubated for different times at 37° C in a 5% CO_2 atmosphere.

In some culture, phytohemoagglutinin (PHA) was added 1 hour after seeding; other samples were, on the other hand, cultured in the absence of PHA. At different time intervals after the beginning of culture, ^{3}H-uridine (New England Nuclear Co., specific activity 2 Ci/mM) at the dose of 20 uCi/ml was added for 20' to the culture medium. After this interval the slides were washed three times in a non radioactive medium, fixed in ethanol 70% and then in water and autoradiographed using NTB2 liquid emulsion. Cultures in universal containers. The composition of culture medium was the same as with the culture in Petri dishes. At different intervals after PHA addition, the cultures were labelled for 20' with 20 uCi/ml of ^{3}H-uridine. At the end of incubation period, the cells were centrifuged, suspended in non-radioactive medium containing an excess of cold uridine to arrest the incorporation, and centrifuged again. The pellet was dissolved in distilled water and treated with TCA to precipitate the RNA. The precipitate was collected on millipore filter and the filtrate was counted with a liquid scintillation counter.

Results. The acid phosphatase and peroxidase activity was studied in control lymphocyte cultures and in cultures stimulated with PHA, both from control individuals and from professionally exposed workers. These enzymatic activities were rather low in unstimulated lymphocytes. As early as 15 hours after addition of PHA, numerous granules showing acid phosphatase and peroxidase activities were seen. The number of grains increases in cultures incubated for 20 and 30 hours. The modifications of the enzymatic activities were observed in a percentage of cells ranging from 40 to 55. No difference in enzymatic activity was observed in control individuals and in workers professionally exposed.

The results of the autoradiographic experiments with ^{3}H-uridine indicated an increase both in the percentage of labelled cells and in the number of silver grains per cell. In the control cultures without PHA, the percentage of labelled cells was low, between 10 and 20%, and the cells were only very slightly labelled. After addition of PHA the percentage of labelled cells rose to 30 and 55% 2 and 4 hours after stimulation, respectively. No further increase was detected 8 hours after PHA. The number of silver grains over the nucleus increased rapidly during activation up to 2 or 3 folds over the control value.

The biochemical results are consistent with the autoradiographic data. The incorporation of ^{3}H-uridine was rapidly activated after stimulation with PHA; it rose rapidly between 1 and 6 hours following PHA administration up to 8-10 folds the control value and remains constant.

The nature and the biological role of the RNA synthesized in the stimulated lymphocytes is unknown. Some of it may be related to the process of DNA synthesis and cell proliferation wich commences later, but it probably reflects also an increase of the portion of chromatin which is active in transcription. This conclusion is consistent with the morphological observations showing that the amount of euchromatin versus heterochromatin increases significantly in the transformed lymphocytes.

Contracting party of the Commission :
EUROPEAN LATE EFFECTS PROJECT GROUP (EULEP)
Contract number : 092 - 72 - 1 BIO C
Chief of the research group : C.F. HOLLANDER
General object of the contract :
PERFORMANCE OF A CO-OPERATIVE RESEARCH PROJECT ON LATE SOMATIC EFFECTS OF IONIZING RADIATION IN MAMMALIAN ORGANISMS.

EULEP has pursued its efforts during the year 1974 on

a) standardization of experimental conditions in the participating institutions.
b) coordination of the planning and performance of on going research projects in the area of radiation late effects, and
c) on execution of specific cooperative projects on carcinogenesis, on dysplasic and dystrophic lesions and on the toxicity of radioisotopes.

STANDARDIZATION COMMITTEES

B-1. Standardization of radiation dosimetry
B-2. Standardization of conditions for animal experimentation
B-3. Standardization of histopathology
B-4. Standardization of laboratory methods.

SPECIFIC COOPERATIVE PROJECTS

B-5. In the field of carcinogenesis
B-6. In the field of the non-neoplastic changes
B-7. In the field of internal radioisotope toxicity.

Results of project N° 1
Head of the project : J.J. BROERSE
Title of the project : COMMITTEE ON DOSIMETRY STANDARDIZATION

Over the past year,members of the dosimetry committee have made site visits to some laboratories in order to assist in specific dosimetry problems (e.g. K.J. Puite, Additional EULEP dose intercomparisons, ITAL, external report no. 15, March 1974). Several improvements in exposure arrangements have been achieved.

A new project on dose distributions for partial-body X-irradiations has been initiated. These studies are essential for investigations of the response of specific organs e.g. lung and oesophagus. Preliminary information is obtained for partial-body irradiations performed at Rijswijk. In the continuation of the project, the various exposure arrangements at different laboratories will be compared and the dose distribution outside the irradiated area will be investigated.

Preparative action has been taken for an European Neutron Dosimetry Intercomparison Project (ENDIP) to be conducted in 1975. The aim of this project is to compare the results obtained by various groups in performing mixed field dosimetry for neutron radiobiology and neutron radiotherapy. The intercomparison will be performed at two locations : GSF, München/Neuherberg (for institutes working on neutron radiobiology and related dosimetric studies) and TNO, Rijswijk (for institutes working on fast neutron radiotherapy). Some institutes cooperating within EULEP have already expressed their interest to participate in ENDIP.

Results of project N° 2
Head of the project : A. DUNJIC
Title of the project : COMMITTEE ON LABORATORY ANIMALS STANDARDIZATION (CLAS)

CLAS meetings

A first meeting was held in Louvain (December 10 and 11, 1973) to define Clas activities in 1974 as well as to initiate longevity studies.

In addition, several small meetings were held in 1974 at some participating laboratories with the coordinators and/or committee members to initiate interlaboratory coordination of various aspects of longevity studies (Reisensburg, May 3, Rijswijk, September 5, Oxford, September 20, München, September 24).

At a meeting in München (September 24) the Pathology Committee discussed standardization procedures with respect to further collaboration on pathological aspects on longevity studies.

Finally, the annual CLAS Training Meeting was held at Sundbyberg (November 29). The scientific part of this meeting was devoted to the "Handling of internal emitters in animal experimental work". The communications will be published in the EULEP Newsletter. During the administrative session of the meeting, the progress reports on the CLAS studies going on in participating laboratories were presented by the coordinators and the committee members.

Longevity studies

Fifteen participating laboratories initiated coordinated longevity studies and provided information on animal strains utilized for CLAS in 1975.

Five laboratories communicated previous longevity data for comparative analysis by the life table method.

A draft on minimal requirements concerning pathological observations was sent to all laboratories.

Lipid content of 4 diets used in 4 laboratories (Liège, two Rijswijks and Louvain) were analysed. In the same laboratories, long term observations are carried out on experimental animals receiving new or special diets.

Biotyping assays are advanced in the Rijswijk's laboratories and bacteriological controls are carried out in 4 other institutions (two Münchens, Mol and Ulm).

LD 50/30 studies

The 3rd progress report, which represents a synthesis of the data already collected on a voluntary basis during the last three years was presented at the Sundbyberg meeting by Dr. Metalli. Mouse data from 5 laboratories are sufficiently advanced to draw preliminary conclusions and formulate concrete recommendations for further activities in this field.

Other activities

A first listing of mouse and rat strains in EULEP laboratories was published this year.

A list of selected references on long term animal observations and their statistical analysis was communicated to all EULEP laboratories.

The proceedings presented at the first Clas Training Meeting in Louvain were published in the 1974 May issue of EULEP Newsletter nr. 6.

Results of project N° 3
Head of the project : W. GÖSSNER
Title of the project : COMMITTEE ON PATHOLOGY STANDARDIZATION

In 1974 two workshops have taken place in Münich.
The first workshop was held on May 4-5, 1974 with the main topic "Neoplastic and non neoplastic lesions of the thyroid".

Thirty one selected cases of thyroid lesions in rats, mice and mastomys were presented and discussed.
These cases included examples of the following lesions :
Hyperplastic nodules (dysplasia) and adenoma. C-cell hyperplasia.
Follicular carcinoma. Papillary carcinoma. Undifferentiated carcinoma.
Small cell carcinoma.
Medullary carcinoma.
Focal lymphocytic thyroiditis.

Lectures by Dr. Boorman and Dr. Walinder were given on "Morphological and functional aspects of medullary thyroid carcinoma in the rat", and on "The development of radiation induced neoplasia in the thyroid gland in mice" respectively. In the final discussion on classification of thyroid tumors, the members decided to apply also,as far as possible,the new WHO-nomenclature (Hedinger) for thyroid tumors in experimental animals.

The second workshop was held September 23-24, 1974 with the main topic "Neoplastic and non neoplastic lesions of the lung".
Dr.H.L. Stewart (Registry of Experimental Cancers; National Cancer Institute, Bethesda / U.S.A.) gave a lecture on "Tumors and non neoplastic proliferative lesions of the lungs of mice".
This lecture was based on 42 cases, which adduced examples of the following lung lesions :

Alveologenic tumor (solitary and multiple, mixed glandular, undifferentiated cell and spindle cell pattern). Transplants of alveologenic tumors.
Adenomatosis.
Squamous cell carcinoma.
Squamous cell metaplasia. Malignant hemangioendothelioma.
Microscopic slides of these cases were distributed to all member institutes and will be kept in the registry of the committee.

In addition 41 cases of lung lesions in mice and rats were presented by members of the committee. These cases included examples of the following lesions :

Alveologenic tumor / carcinoma. Pulmonary adenomatosis.
Squamous cell metaplasia. Mesothelioma. Metastasis of carcinoma of the Harderian gland. Metastasis of hepatocellular carcinoma.
Reticulum cell sarcoma type B. Peribronchial and perivascular spread of lymphosarcoma. Peribronchial and perivascular lymphoreticular hyperplasia.
Alveolar desquamative pneumonia, histiocytic type with crystalloids.
Alveolar and interstitial pneumonia, histiocytic type. Chronic pneumonia.
Endangiitis pulmonalis.

Dr. Mass demonstrated lung tumors in rats produced by inhalation of radionuclides including light and electronmicroscopic observations.

The following nomenclature of the lung lesions in mice and rats has been discussed :

Mouse

Alveologenic carcinoma
Squamous cell carcinoma
Malignant hemangioendothelioma
Adenomatosis
Squamous cell metaplasia
Lymphoreticular tumor (primary and secundary)
Mesothelioma
Sarcoma

Non neoplastic : Pneumonia, histiocytic type
Granuloma (Fungus)
Fibrosis

Rat (classification proposed by Dr. Mass)

Bronchiogenic carcinoma

Squamous cell
Adenocarcinoma
Mixed type
Undifferentiated (all the characteristics of basal cells)
Large cells anaplastic
Other types (clear cells etc.)

Bronchiolo-alveolar carcinoma

Bronchiolar type (60% ciliae and goblet cells)
Alveolar type
Mixed type

Sarcoma

Mesothelioma

In addition both workshops presented and discussed several problem-cases sent to the consultation centre.

Results of project N° 4
Head of the project : A. KEYEUX
Title of the project : COMMITTEE FOR CLINICAL PATHOLOGY STANDARDIZATION

The Committee was asked to cooperate in 4 projects of standardization and coordination.

1. The supply of ALS and ALG which was necessary for the DIMS project
2. The development and the standardization of an immune monitor system in the mouse.
3. The development and standardization of a computer method for the analysis of isotope radiocardiogram.
4. The development of a method for the determination of elastine in the lung.

Development and standardization of a computer method for the analysis of isotope radiocardiogram.

The time concentration curve recorded by external counting of the radioactivity of the precordial region after intravenous injection of a radiotracer, is characterized by two peaks followed by a plateau. Taking into account that the pulmonary blood vessels join the right and the left heart, it appears that in relation to the pulmonary blood circulation the single dilution curve corresponding to the first peak could be treated as an input function and the other single dilution curve corresponding to the second peak as an output function.
Nevertheless, the great variability in shape of the radiocardiogram in the rat, makes the fitting by a theoretical curve based on a two compartment model difficult. A more adequate approach was found by using the differential extraction of Tc99m pertechnetate and I131 antipyrine in the lung region. The difference obtained between the isotopic radiocardiograms performed with both radiotracers, characterizes the permeability of the lung microcirculation.
In conclusion, the first method can be used only in typical radiocardio-

grams, where it can yield information on the pulmonary blood flow and blood volume. Whereas, the second method while simplifying the analysis of the results is limited to an assessment or vascular permeability.

Development of a method for the determination of elastine in the lung.

Elastine is characterized by two specific amino acids which are present, however, only in very small quantities (<.05%). Since changes in elastin could accompany late effects in lung, it was attempted to develop procedures to determine desmosine and isodesmosine in extracts from rat lung. The following experiments were carried out :
Determination by column chromatography is suitable for isolation of desmosine, but experiments published in the litterature have worked with amounts in the order of mg rather than µg. We developped a microcolumn system in which determination is carried with flurame a new fluometric reagent for amino acids. Desmosine could be thus determined in 1 mg of pure elastine, but an improvement in sensitivity is still needed to determine it in lung. High voltage electrophoresis was succesful also only in amounts greater than would be available.
Gaschromatography : Trimethylsilylation of desmosine yielded several products one of which was lysine either a contaminant or a breakdawn product. Esterification with subsequent trifluoroacetylation did not give a derivative which could be separated on the columns available.
Unfortunately desmosine and isodesmosine are not commercially available. A gift of about one mg has been used up in these studies and we have now isolated a new batch with which we will continue the development of the method during the next year.

Results of project N° 5
Head of the project : H. SEIDEL
Title of the project : COMMITTEE ON CARCINOGENESIS

A. Meetings

During 1974 the activities of the group increased in many respects. The information exchange between the laboratories was improved mainly by means of 4 meetings, that were attended by most laboratories.

B. Scientific report

1. Experiments on leukemia induction at the cellular level in mouse syngeneic radiation chimeras.

This is a combined effort of Metalli/Rome and Coggle and Lindop/ London. Rome follows the dose dependence of the development of lymphomas and reticulum cell sarcomas whereas London studies myeloid leukemia. It confirmed that more systemic tumors arise in lethally irradiated recipients transplanted with preirradiated donor cells than with non irradiated ones. A dose response study showed that the total incidence is increased and the latency period shortened only after doses above 400 Rad. A first estimate of the radiosensitivity of the potentially malignant cells yielded about 130 rads for the D_0. The effect of cell killing as a factor in the decrease of the induction curve of leukemias is carefully considered and special attention is given to the number of CFU-s transplanted. Special stem cell studies (CFU_s and CFU_2) after radiation exposure are performed in London with emphasis on the characterization of the target cell for leukemia induction. The study of Gong et al. on the role of bleeding on the incidence as well as on the latency periods in radiation induced leukemias could not be confirmed.

2. The role of the immune system during Carcinogenesis

2.1. DIMS. The development of suitable immune monitor systems has combined 5 different laboratories : Doria (Rome), Flad (Ulm), Jovanovic (Louvain), Sassen (Mol) and Van Bekkum/A. Kruisbeek (Rijswijk). The Rijswijk group acted as coordinator and supplied the subgroup with a standard ALG preparation (coded E2). The Rijswijk group investigated the effect of long-term ALG treatment on the cellular and humoral immune response in mice. Various schedules were used, all attempting to economize ALG, by combining ALG treatment with thymectomy. Flad concentrated on the T-cell response of peripheral blood lymphocytes, Doria studied the spleen cells using the Mishell and Dutton technique, Jovanovic used rats cytotoxic activity of splenic lymphocytes after shortterm as well long-term administration of ALG.
The results will be published in detail in the near future.
At present it can be concluded that a good immune suppression is achieved in mice by this ALG preparation only up to about 3 weeks. Furthermore there is still an urgent need for monitor tests that give consistent results in mice, and the subject is further complicated by the effect seen after administration of normal horse serum. On the contrary, the situation in rats seems to be satisfying as results of Jovanovic and of Rijswijk show. A very good scheme in rats is worked out now, and the role of the immune system during carcinogenesis in this species can now be studied in different models using animals with immune depression maintained for long periods.

2.2. Tumor induction in the liver by administration of colloidal gold is studied by Jovanovic.
The bladder tumor induction by MNU is studied by Bolhuis and Kruisbeek. Tumors either spontaneously arising or induced have been transferred to "in vitro" culture to serve as target cells for the tumor specific immune response. The general immune response

was measured in an induction study using DIMS methodology.

Conclusions

The committee has increased its activities during 1974 and formed a good basis for further development of its programme. Several new projects were presented by the members that fit into the outlined programme but could not be funded by the EULEP. The committee hopes very much that its financial basis will be improved in the coming year.

Results of project N° 6
Head of the project : G. GERBER
Title of the project : COMMITTEE ON DYSPLASIA AND DYSTROPHIA QUANTITATIVE AND QUALITATIVE CELL CHANGES.

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1. Late effects of irradiation on the CNS as a model for late vascular changes.

The group, consisting of 11 scientists from 5 different laboratories, is investigating the influence of irradiation on the rat brain, with special emphasis on the vascular supply. The eventual aim is to elucidate the tissue components that are dose-limiting in radiation therapy. Mature rats were irradiated under standard conditions in 3 participating laboratories. The dose of X-rays (2000 rads) was chosen to be in the clinical "treshold" range of tissue tolerance.
Some 32 different determinations in relation to the physiologic and, in part, morphologic response of the rat brain are being followed - if possible - over a period of 2 years. Such a long "latent" period is essential in order to allow late effects to develop.

Preliminary results, covering the first year after irradiation are now available. Some investigations have already reached the 18 month-mark. At one year after irradiation, several biochemical and physiological changes have been found to occur, while no striking differences could be observed in the cell kinetics, ultrastructure and angio-architecture of the rat brain.
The most important physiological changes observed are : an increased serotonin content of the brain, a transient increase in DNA content at 9 months, and a decreased cerebral blood volume. The oxygenation of the cortex appears also to be higher than in the controls after irradiation.

The cell proliferation of the subependymal cells has been found to decrease dramatically over the period of one year. No significant changes, however, were observed in the labelling indices of the endothelial cells, and of white- and gray matter oligondendrocytes. After the dose of 2000 rad no consistent changes in the permeability of the endothelial cells, as assayed by a quantitative Electron Microscopy technique were seen. Higher doses however, yielded obvious changes. Interestingly, Electron Microscopy investigations showed that the neurons seem to undergo repair, as indicated by signs of synthesis of endoplasmic reticulum.

In conclusion, at one year after irradiation with 2000 rad, no striking differences were observed in the cell kinetics, ultrastructure and angio-architecture of the vascular system of the rat brain. There were, however, a number of biochemical and physiological changes. Some preliminary observations at 20 months with the thick-section techniques revealed, however, very obvious changes in putamen, globus pallidus and frequently in the hippocampus.

It seems at present that physiological and biochemical changes preceed anatomical changes and it will be worthwile to place special emphasis on (micro) physiological changes in these areas.

2. Late effects in lung

The project was initiated in 1973 as a cooperative biochemical study of Warshaw and Mol and completed in 1974 by London and Louvain. The project intends to elucidate the conditions and mechanism under which late effects (fibrosis) develop after radiation therapy of the thorax. It also is concerned about induction of tumors in the lung after localized or general irradiation. An intercomparison program planned for 1974 to evaluate differences in strain and radiation conditions could not be achieved due to technical difficulties at the radiation units.

Nevertheless, the conditions of radiation were agreed upon by the group and have been followed during the past year.
The following results were obtained.

Biochemical endpoints (Dancewicz, Gerber)

A second series of rats was irradiated with 3KR. They extended the data obtained in the earlier group : during the early period changes in enzymes 'acid phosphatase, cathepsine and fibronolytic activity) are most noticeable reaching a maximum around 6 weeks after exposure. Later, an increase in collagen and changes in peroxides and peroxidative activity become visible.

Morphological endpoints (Maisin)

Adult BALB/c mice were exposed on the right hemithorax to increasing doses of X-rays (500, 1000, 2000 or 5000 R of X-rays). The mice will be killed at various periods from 15 minutes to 24 months after exposure. Changes in the ultrastructure of irradiated lungs and the permeability to horseradish peroxydase of the alveolar capillary membrane after X-irradiation are studied. After a single exposure to 2000 R or 5000 R of X-rays three phases with respect to the ultrastrcutural changes in the mouse lung may be distinguished : the early or exsudative phase, the intermediate or proliferative phase and the chronic phase. Already a few hours after exposure focal lesions are detectable in all types of lung parenchymal cells as well as in the plasma membrane, the basement membrane, the interstitium and the capillaries. The analysis of the results obtained after lower doses of irradiation is in progress in view to determine the exact part played by the epithelial cells and the endothelial cells in the development of radiopneumonia.

Physiological endpoints (A. Keyeux, D. Jovanovic)

During the past year, series of 10 to 20 rats were irradiated with 500, 1000 and 1500 R on the thorax.
Changes in ventilation were tested by comparing ^{133}Xe clearance in

the irradiated animals and their unirradiated controls. Net reduction of the regional ventilation per unit of volume were observed in the well ventilated compartment during the acute phase (about 1 month after irradiation) and in the poorly ventilated compartment during the late phase (about 1 year after irradiation) for every dose used. The late effect of thorax irradiation on the comparative distribution of ^{99m}Tc pertechnetate and ^{131}I antipyrine in the lungs is currently under investigation.

Cancers of the lung (P.J. Lindop)

The lung tumour incidence in mice following exposure to whole body irradiation has been investigated and found to depend upon the strain of mouse and their age at exposure.
The influence of urethane and radiation has also been studied in this laboratory with respect to tumour induction in the lungs of mice.
In an attempt to elucidate the mechanisms of lung tumour induction a study of lung cell kinetics was undertaken. It was found that the tritiated thymidine method employed disturbed the cell kinetics of the lung so that it was difficult to calculate normal cell cycle times. The bronchiolar epithelial cells showed a longer term disturbance due to incorporated radioisotope than the alveolar epithelial cells.
The proliferation of cells after whole body exposure to external irradiation was studied and different responses were again found for the alveolar and bronchiolar populations. It seems likely that the two cell populations have separate feedback control mechanisms of differing radiosensitivities.
The study of cell kinetics has led to some insight into the mechanisms involved in lung tumour formation. It is proposed that in the future the present knowledge should be extended and that high levels of incorporated tritium should be added to the list of perturbating agents.

Results of project N° 7
Head of the project and coordinators : P.J. LINDOP
B.E. LAMBERT (Tritium and its compounds)
W.A. MÜLLER (Bone-seeking isotopes - EBONY)
Title of the project : POINT SOURCE EFFECTS COMMITTEE

A. Tritium and its compounds

1. The tritium cancer induction experiments were continued. Mice were exposed in utero to ^{3}H incorporated in their DNA (from continous infusion of tritiated thymidine) and tumours were scored as they develop in these irradiated mice and controls. Aim of the experiment is to provide dose linked information about cancer induction in mitotic and post mitotic tissues.

2. Shorter term experiments are being carried out on some of the totally labelled mice produced as (1). These experiments involve (a) cell kinetic studies in various tissues at various times; b) studies of cell death and chromosome aberration frequency in oocytes and spermatogonia.

3. Similar comparative experiments as in (2) involving the use of HTO as the irradiating medium.

4. Development of links with other groups in EULEP to perform collaborative work :

   a) Joint project between Abteilung für Klinische Physiologie, Universität Ulm (W.Schreml) and Barts (B.E. Lambert) aimed at investigating the performance of the mouse long lived bone marrow stem cell (bone marrow lymphocyte) when triggered into proliferation after irradiation with tritium.

   b) Similar experiment as in (a) utilising the functional capacity of the hepatocyte after partial hepatectomy.

c) Provision of totally labelled animals for the lung project (for both non-neoplastic and neoplastic changes)/

d) Possible provision of totally labelled mice for the study of the regenerating capacity of irradiated endothelial cells.

B. Bone-seeking isotopes - EBONY

On the whole, the results for all the three radionuclides Ra, ^{223}Ra, ^{239}Pu and ^{20}Sr tested show an unexpectedly good agreement of the retention data for the different institutes. Though the different techniques environment, animals etc. differed largely in the intercomparison results, this fact showed not up by systematic differences. Thus the participants realized that "we speak the same language" in these incorporation studies. Consequently there seemed no need for supplement or new retention studies in the same way.

Experiment on intercomparison of ^{223}Ra behaviour.
A test-solution was produced and distributed by the Neuherberg institute. This solution possessed the same characteristics as the final injection solution. $^{223}Ra(NO_3)_2$ was diluted in physiologic NaCl-solution. pH 4.5-6.5, specific activity ca 10 µCi/ml. Each laboratory was requested to develop optimum conditions for measuring the activity with respect to the later measurement of biologic samples.
A γ-measurement was recommended using a Na-I well-hole crystal within an energy range from 0.200-0.600 MeV. Calibration and determination of efficiency was to be performed.

The final experiments were then carried out according to the instructions of the minutes of the First EBONY-Meeting (Brussels, April 12-13, 1973) EULEP-Newsletters Nr. 4, 1973.
The amount of injected activities differed to a considerable amount in the individual institutes because different time schedules were

needed and because errors in diluting the injection solution had to be avoided. The proposed injection of 30 µCi/kg was performed in Neuherberg. The values for the other institutes were : Mol 69 µCi/kg, Harwell 40 µCi/kg, and Warsaw 42.7 µCi/kg.

For future radium incorporation studies the long-lived isotope ^{226}Ra was proposed (see report of the 2nd EBONY-Meeting, Neuherberg October 10-11, 1974)

Contractor: International Commission on Radiological Protection

Contract No: 091-73-1 BIOC.

Head of research team: C.G. Stewart, Chairman, ICRP.

General subject of contract: Development of fundamental data on radiation exposures and the establishment of recommendations regarding maximum permissible exposures.

Brief general description of the work carried out:

During 1974 the Commission met to review the work being performed by its committees and task groups (see Part B). It also decided to institute a complete revision of its recommendations, previously issued in 1966 as ICRP Publication 9. The process of preparing the new recommendations is expected to take two or more years.

No reports were published by ICRP in 1974. It is expected that the report on "Reference Man" will be published in early 1975.

ICRP committees and task groups worked on the following topics in 1974:

Biological effects of inhaled radioactive particulates.

The radiosensitivity of the embryo and foetus.

The influence of factors such as LET and protraction of exposure on genetic and somatic hazards.

The quantification of the severity of radiation effects for the purpose of estimating detriment.

Dosimetry of radionuclides within the body (a revision of ICRP Publication 2).

The hazards of Radon, Thoron and their daughter products.

Respiratory absorption and elimination mechanisms.

Protection of the patient in radiotherapy.

A revision of ICRP Publication 5.

Emergency and accidental exposures.

Radiation protection in uranium mines.

Releases of radioactivity into the environment.

The membership of the Commission and its committees is unchanged from 1973.

Committees 2, 3 and 4 met during 1974 to review their work and to prepare reports to be submitted to the Commission in 1975.

GRUPPE BIOLOGIE ISPRA
KOMMISSION DER EUROPÄISCHEN GEMEINSCHAFTEN

BIOLOGY GROUP ISPRA
COMMISSION OF THE EUROPEAN COMMUNITIES

GROUPE DE BIOLOGIE ISPRA
COMMISSION DES COMMUNAUTES EUROPEENNES

Biology Group Ispra-Italy

Head of the research team(s): C.MYTTENAERE

General subject of Contract: Direct participation of the Commission in its established programme

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Brief general description of the work carried out:

The research work carried out in 1974 was pursued according to the established programme in close connection with various EURATOM Associations or Group Contracts. Its principal headings are:

1. Environmental contamination and radiation effects.

As in the past, part of the activities were devoted to specific aspects of the cycles of various radionuclides in the food chain as well as in terrestrial and aquatic systems. As usual, these works were carried out in collaboration with the Association EURATOM-CEA, Association EURATOM-ITAL and the Joint Research Center.

The investigations of chemico-biological interactions, although reduced during 1974, have given useful information on the transfer of radioactive metals. In particular, the studies dealing with the binding of metals with proteins were carried out in collaboration with the Joint Research Center.

As a partial contribution to the Integrated programme on "Radioentomology", the studies were mainly concerned with various physiological effects of ionizing radiations and/or chemical substances on different insect species; and part of the activity was devoted to the development of adequate rearing facilities. The research work was performed in close collaboration with the University of Padova/Italy.

2. Genetical Biochemistry. DNA damage by radiation and mutagenic chemicals Mammalian mechanisms involved in the enzymatic expression and the repair of this damage.

The activities are included in a pluriannual effort carried out in the framework of the Euratom Programme Group "Biochemistry of DNA damage and

repair". Part of the work was carried out in close cooperation with Institutions engaged in the researches sponsored by this Group, namely: Biochemisch Laboratorium, Rijksuniversiteit Leiden, Laboratorio di Genetica Biochimica ed Evoluzionistica di Pavia; Laboratoire de Biophysique et de Radiobiologie de l'Université Libre de Bruxelles; Centro d'Enzimologia del CNR e Istituto di Chimica Biologica dell'Università di Roma.

The project has a dual aim: first, to investigate the DNA lesions induced by ionizing radiations; second, to characterize the mammalian enzymes involved in the repair or in the direct expression of the molecular damage induced in the genetic material.

The experimental work makes a large use of polydeoxynucleotides which may serve as a convenient DNA-like molecules for testing enzymes using DNA templates and DNA substrates. These products are largely distributed also to outside laboratories.

3. Radiation structure of ionizing radiations in tissue and its relation to their spectral energy deposition in biological structures and to the biological effectiveness.

This activity is part of the integrated long-term programme of the "European Dosimetry Group" (see reports in sector "Dosimetry"). It comprises two projects:

- Radiation structure in biological material and in model substances: it is the scope of these studies to provide the basic physical data necessary for dosimetry and microdosimetry, like e.g. W-values, ranges, mass stopping powers, and track structures of charged particles in tissue equivalent materials.
- Evaluation of the energy deposition of different types of radiations to small biological volumes and its relation to the corresponding biological effectiveness: energy deposition spectra and the average energy depositions in small radiosensitive biological volumes represent a good characterization of radiation quality and play an important role in the discussion of the relative biological effectiveness at very small values of absorbed dose. Therefore, this project is mainly concentrated with experimental and theoretical evaluation of energy deposition spectra of 200 KV X-rays, fast neutrons and their secondaries in biological tissue, and with their relation to biological effectiveness.

Results of Project No. : 1

Head of Project and scientific staff: C.Myttenaere, A.Berg, R.Cavalloro, G.Delrio, P.Guillot, E.Levi, M.Merlini, G.Premazzi, O.Ravera, P.Reiniger, P.Scoppa; S.D.Gerking*; M.Cavelier**, P.Dabin**, W.Penning**, J.Y.Standaert**, K.Strijbis**

Title of Project : Environmental contamination and radiation effects.

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1. Transfer of radioactive and associated pollutants in food chains.

a) Transfer in terrestrial ecosystems

Direct contamination studies of pure strands of clover and rye plants were made under greenhouse and growth room conditions to ascertain the retention of Iodine and Strontium when applied as NaI and $SrCl_2$ at a rate of 4 mm/h for one hour. The plants were harvested after 1, 3, 24, 72 and 168 h to determine the residence time of these elements. Another set of plants was treated (24 hrs after start) with an equivalent amount of water or with the same treatment.

Results indicate that for clover the percentage of I retained was almost constant ($\pm$ 25% in the period 1 - 168 h); Sr retained was higher but decreased with time to 60%. For rye, the I retained was much less (8%) and Sr was also less and relatively constant.

Calculated half-lives seem to agree with those in the literature (5 - 20 d). Washing decreases the I and Sr left on clover and rye; a second similar spray did not change the I retained but slightly increased the Sr.

Bean plants grown in controlled conditions were treated with small drops of a known volume and harvested after 1-168 h. An increase in the concentration of applied I-131 was not followed by a proportional increase in uptake. In the presence of thiosulfate losses after 15 d were as high as 70% while in its absence they were 25%.

The presence of $SrCl_2$ in the treatment solution seems to decrease the penetration of the I; the I, however, did not influence Sr penetration.

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* Visiting Scientist

** Post-graduate students

Studies on the antagonistic effects of Na, K and Cs on Zn uptake (carriers were used at a concentration of 1 mM) show that the washing and exchangeable percentages of Zn are clearly higher in the presence of these elements.

A marked decrease of Cs-137 penetration was noted when Zn^{++} was present at concentrations higher that 10^{-7}M.

Preliminary work on the evaporation of radioiodide from soils was continued. Carrier free I-125 and I-131, with and without reducing agent, were evaporated from filter paper discs in a pH range from 1.1 to 11.6 under fluorescent, ultraviolet and infrared light. Evaporation losses were highest under UV light and at a low pH and reached in 24 h 35% of the amount applied. In a prolonged exposure under fluorescent light and at a pH of 2.5, 20% were lost in the first 24 hours, the total loss in 7 days attaining 55% of the amount supplied. The addition of stable iodide in concentrations found in rainwater markedly increased the evaporation velocity of radioiodide.

The uptake of Cr-51 by rice plants from a Cr-contaminated soil was studied in relation to the chemical form of chromium (trivalent and hexavalent), the method of irrigation (flooding and intermittent irrigation) and the growth stage (tillering, flowering, maturity). The soil had been contaminated uniformly by spraying 100 mg/kg of Cr marked with Cr-51.

The uptake of Cr, measured as Cr-51, by the rice plants was limited to the roots and collars. Concentrations in the plants were higher in dry than in the wet treatment and, more important, for the soils with Cr-VI than with Cr-III.

The addition of Cr-VI to the soil significantly increased the dry weight of the experimental plants in the wet treatments but no explanation of this effect can be given at present.

The absorption of Zn and Cd by irrigated rice was studied in controlled conditions (water culture) in irrigated rice field "models" and "in situ" (experimental station Vercelli):

- The Cd and Zn toxicity levels were first determined in nutrient solution (Hoagland) containing Cd and Zn concentrations up to 2 ppm. Statistical analysis of dry yields showed that the concentrations normally used in these conditions have to be inferior to 0.10 ppm for Cd and 1.00 ppm for Zn.

- The influence of stable Cd on the absorption of Zn was studied in nutrient solutions traced with Cd-109 (stable Zn concentrations 0.0; 0.1; 0.5; 1.0 and 2.0 ppm; stable Cd concentrations 0.0; 0.01 and 0.1 ppm). Stable Zn, stable and radioactive Cd will be determined in the different organs of the rice plant. The contribution of the roots and of the shoot basis on Cd absorption was estimated for growing young rice plants (one month) in Cd-109 traced nutrient solution or irrigated water (double containers) during 4 days. Cd absorbed by the roots is mainly concentrated in the radicular system, the concentration factor varying with the Cd level (800-300 for concentrations of Cd stable from 0.25 to 2.00 ppm). Part of the Cd absorbed by the shoot basis is translocated to the roots and the transfer water-shoot is of the same order than between nutrient solution-shoot (40-8 for the same concentrations).

- The transfer of Zn-65 from water to rice was studied in irrigated rice field models. Analysis of data obtained in 1973 showed that the pollution of irrigation water with Cd ($5\ 10^{-3}$ppm) enhanced the Zn-65 translocation and consequently the caryopsis activity. The caryopsis of the Cd irrigated plants contain three times more Zn than those of the plants growing on soil flooded with non polluted water. A second experiment was realized in 1974 in the same installations (irrigation water: 1 ppm stable Zn) to study the influence of stable Cd levels ($2.5\ 10^{-3}$ppm; $5.0\ 10^{-3}$ppm and $50\ 10^{-3}$ppm) in water on Zn-65 absorption. Zn-65 content of the plant does not decrease proportionally to the water stable Cd content; such a result can find a partial explanation in the decrease of water activity due to a higher migration of Zn in the soil when Cd concentration increases.

- Samples of water, soil and plants were collected in the field (Vercelli) during all the vegetative cycle and at harvest in order to determine the actual stable Zn and Cd content of the different compartments of a rice field ecosystem fertilized with different N and P_2O_5 levels.

b) Transfer in aquatic ecosystems

- Zn-65 and Sr-85 accumulation by freshwater algae

Monospecific axenic culture of Selenastrum minutum (Näg) Collins (modified PAAP medium without any chelating agent) was contaminated with 0.2 µCi/ml of Zn-65($ZnCl_2$) as well as Sr-85($SrCl_2$)-(the concentration of stable Zn was 7.00 ppb). Batch cultures were constantly swirled and maintained at 23 ± 2°C under a constant fluorescent light (3000 lux). Initial population density was 10^4 cells/ml. The experiment was carried out for 13 days and the concentration factor was 496 ± 13 for Zn-65 and 1068 ± 120 for Sr-85. For Sr-85 the equilibrium between the activity of the algae and that of the medium was reached on the 6th day, whereas for Zn-65 there was a linear increase in activity until the end of the experiment. A certain part of the measured activity was probably adsorbed in the cell membrane. For our purpose this was not important for two reasons: 1) zooplankters accumulate the adsorbed as well as the incorporated radionuclides with their food; 2) the amount of activity released by the algae to the uncontaminated medium was negligeable, indeed, after 14 days, the activity released was about 15% for both Zn-65 and Sr-85.

- Transfer of Zn-65 and Sr-85 from algae to Daphnia hyalina

Three experiments were carried out to measure the filtering rate of Daphnia hyalina and the transfer of Zn and Sr. For each experiment about 100 Daphnia were maintained in filtered pond water at 23 ± 2°C in the light; they were fed S. minutum cultured in a radioactive medium (modified PAAP medium + 0.2 µCi/ml Sr-85 and Zn-65).

The concentration of algae in the suspension was about 10^6 cells/ml. The calculated values of the feeding and the filtering rates were essentially the same for Zn-65 and Sr-85. The mean filtering rate being 0.24 ± 0.10 ml/individual/hr, and the radioactivity content of the algae living in 1 ml of water considered as equal to 100, 24% of their activity is taken in by Daphnia in one hour.

These results give a rough idea of the transfer of radionuclides from phytoplankton to zooplankton in natural conditions for two reasons: 1) the density of the Daphnia population was similar to that reported for the natural environment and 2) the short duration of the experiment and the long adaptation time probably combine to reduce the effects of experi-

mental conditions. On the other hand our results may be overestimated because the density of the algal population used in our experiments was higher than that commonly observed in a lacustrine environment.

Ten groups of 100 Daphnia were kept at 23 ± 2°C in the light and fed S.minutum labeled with Zn-65 (algal population density 7.10^6 cells/ml).

At various intervals of time the activity of Daphnia was measured, and it was found that uptake was rapid, but on the 7th day equilibrium was not reached yet. Loss was also rapid and on the 7th day in nonactive water they had only 6% of the activity accumulated in 7 days.

- Transfer of Zn-65 from Daphnia to fish

Only preliminary results on Zn assimilability by fish ingesting Daphnia have been obtained.

- Transfer of Cs-134 from an organic sediment to an insect larva

This transfer has been studied on a chironomid larva at various temperatures (10, 15 and 20°C). The equilibrium is reached after 40 to 70 hours with a CF (relative to supernatant water) of 12.6 to 16.5, according to temperature. The radioactivity is absorbed mostly (90%) from the ingested sediment. The sediment intake by the larva could be estimated as about 10% of the body weight per day.

- Comparison between Zn and Cd in fish

In view of the well known competition between zinc and cadmium for the sites of exchange in the organism, a comparison must be made between the parameters of accumulation of both elements. This comparison was made for different fish species using Zn-65 and Cd-109 as tracers, together with results obtained from previous research on zinc metabolism. The comparison indicates the following points:

- The accumulation of Cd proceeds at a much higher rate than zinc exchange. Actually while the latter varies in a wide range according to the experimental conditions (from 0.3% per day for Carassius auratus fed a subsistence ration, to 4.8% per day for young Alburnus alborella in growing conditions), the rate of Cd accumulation, for the same two species, reaches 5 to 13% of the body burden at equilibrium, respectively.

- The contribution of direct absorption (i.e. from water) to total accumulation appears to be predominant over intestinal absorption for both elements and especially for Cd (60-80% for Zn, 80-90% for Cd).
- The so-called "concentration factor" (CF, in relation to water) indicates that Alburnus has a capacity of accumulation which is far lower for the non-metabolic element Cd (CF 80 and 300 at 10 and 0.15 ppb Cd in water, respectively) than for the metabolic element Zn (CF around 3000 at 15 ppb Zn in water).
- The concentration of Zn in fish is homeostatically controlled independently of its concentration in the water (50-70 ppm for the two species considered). Cd, on the contrary, is accumulated in fish in relation to Cd in water. The concentration of Cd in fish at equilibrium is actually less than proportional to that in the water. This indicates that fish can employ some internal mechanism in order to inhibit Cd absorption at higher concentrations.
- The threshold at which there is an inhibitory effect of Cd on Zn exchange lies between 10 and 40 ppb Cd in lake water in the presence of a normal Zn concentration (20 ppb). In a simulated rice-field and in the presence of sublethal Zn concentrations (0.2-0.9 ppm Zn), such an inhibitory effect of Cd on Zn exchange could not be detected in the range between 5 and 50 ppb Cd (nominal concentrations).

2. Chemico-biological interactions.

The investigation on the influence of environmental factors on the accumulation of radionuclides and the toxicity of heavy metals to freshwater zooplankton has been discontinued. These studies have shown clearly that several environmental parameters, such as water pH and temperature, dissolved or suspended materials, etc., modify to a considerable extent the accumulation process and the biological effects of heavy metals.

Several techniques for the determination of stability constants of metal complexes have been standardized which include an ion-exchange resin method, pH-titration curves of ligands and their modification in the presence of ligands, activity measurements by specific ion electrodes. The role of cadmium in the reaction catalyzed by triphosphopyridine nucleotide-dependent isocitrate dehydrogenase has been investigated by these techniques.

Further studies are directed to ascertain whether isocitrate or a complex metal-isocitrate is the active substrate of the enzyme.

Studies on the binding of heavy metals to rat liver metallothionein were carried out by radiochemical techniques used in combination with gel-chromatography and gel-electrophoresis. The results suggest that in the native state metallothionein is able to bind Ag and Sn as well as Zn, Hg, and Cu. It was further learned that neither the biosynthesis of the protein nor the incorporation of cadmium are influenced by the presence of the 38 elements studied individually.

The effects of metallothionein on the intracellular distribution of Cd, Hg and Zn in the liver and kidneys of Carassius auratus (goldfish) were investigated. Using their respective radioelements it was found that 24 hrs after injection the following percentages were incorporated into metallothionein:

in the liver:	90%	Cd-109	and in the kidney:	70%	Cd-109
	17%	Hg-203		8%	Hg-203
	25%	Zn-65		2%	Zn-65

A cadmium rich component was isolated from the roots of rice plants. About 95% of the soluble extract was bound to a low molecular weight component of a peptidic nature. Its ultraviolet absorption spectrum suggests an interaction of cadmium with sulphydrilic groups.

The mechanisms involved in the heavy metal-induced impairment of the mixed function oxidase system which metabolizes drugs and steroids have been further clarified. Metal-stimulated peroxidation of unsaturated fatty acids of liver microsomal phospholipids is implicated in the decrease of both drug metabolism rate and cytochrome P-450 concentration. In this connection, evidence has been obtained indicating that inhibition of lipid peroxidation may abolish "in vitro" and "in vivo" the severe impairment of drug metabolism observed in acute lead poisoning.

3. Radiation sensitivity of insects.

Some studies on the physiological effects of gamma irradiation and fast neutrons on Diptera Tripetidae have been started and others have continued. The irradiation was performed at different stages of the life cycle(pupae and adults particularly) of Dacus oleae Gmel. and Ceratitis capitata Wied. Males are more sensitive than females to neutrons (the contrary was observed with γ rays) and the neutron sterilizing doses are clearly lower for males of both species irradiated at pupal or adult stages. Taking as

the end-point the 50% dominant lethals, RBE of fast neutrons with respect to gamma radiations was calculated as 2.05 and 1.66 for pupal and adult stages of Dacus oleae and 1.47 and 1.70 for Ceratitis capitata. As shown by histological studies of gonads of both species, the highest fast neutrons (2979 rads) and gamma radiation (12000 rads) doses completely inhibit spermatogenesis and oogenesis; with the same doses no apparent damage on mesenteron has been observed. Males of both species sterilized with fast neutrons have a sexual competitiveness about 4 times higher than with gamma rays. The neutrons induce mostly genetical effects whereas γ rays disturb cytoplasmic biochemical mechanisms too.

Sub-sterilizing doses have been applied to Ceratitis capitata and their sexual behavior evaluated for different levels of sterility; correlations were established between "inherited sterility" and chromosomic translocations. Competitivity of "substerile males" was estimated and an indirect method of evaluation was worked out expressing the competitivity as a function of more easily measurable parameters, primarily hatching of the eggs; calculated competitivity in the laboratory and in the field, very low for males of Ceratitis capitata, varies between 0.14-0.06 (irradiation of the pupae) or from 0.16-0.03 (irradiation of the adults).

Bioecological studies, using chromotropic traps, γ sterilizing and fluorescent dyes with Dacus oleae adults were carried out in the field.

Studies in "inherited sterility" observed in the G_1 of Gonocerus acuteangulatus Goeze are going on. An irradiation of 6 krad γ rays sterilizes the females which still conserve their mating capability meanwhile the males lose their mating capacity at doses greater than 8 krad without reaching total sterility (Fig. 1). Substerilizing doses provoke a greater inherited sterility in G_1, but this effect decreases in G_2 and G_3 generations. One per cent rate mortality of larvae from eggs layed by substerile insects and a distorsion of sex ratio in favour of the males have also been put in evidence.

Research was also carried out on gamma sterilization of Sitophilus oryzae L.; the total sterilizing effect in males is obtained at 11 krad γ rays.

Part of the activity was devoted to the development of adequate rearing facilities under laboratory conditions (Rhynchota, Diptera, Coleoptera).

Most of these researchs were performed in close cooperation with the University of Padua and represent an integrated part in the Commission's Contractual programme in Radioentomology.

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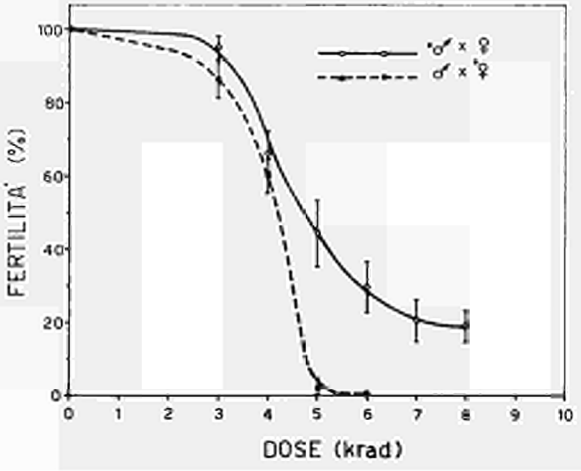

Fig. 1 : Gonocerus acuteangulatus Goeze: gamma irradiation effects on male and female fertility (females reach total sterility at 6 krad without loss of mating capacity; males do not reach total sterility, loosing mating capacity over 8 krad).

Results of Project No. : 2

Head of Project and scientific staff : F.Campagnari, L.Clerici, M.Talpaert

Title of Project : Genetical Biochemistry. DNA damage by radiation and mutagenic chemicals. Mammalian mechanisms involved in the enzymatic expression and the repair of this damage.

- Preparation of polydeoxynucleotides

The production of radioactive and non-radioactive polydeoxynucleotides and their assembling in DNA-like molecules to be advantageously used in nucleic acid enzymology were continued on a large scale. For this purpose, 40 mg of pure terminal deoxynucleotidyltransferase (the key enzyme in the biochemical synthesis of polydeoxynucleotides) were isolated from 20 kg of calf thymus glands.

New types of polymers were prepared for studying the mechanisms of action of the bacterial ATP-dependant exonuclease and of the DNA polymerases functional in DNA repair.

The biochemical work for supplying outside research Institutes with custom-made polydeoxynucleotides and/or with terminal transferase covered 50% of the activity of the laboratory.

- Analytical methods for studying irradiated DNA

Different enzymatic methods for the specific analysis of end-groups in nucleic acids were standardized and combined together as to yield a reliable procedure for the quantitative detection of the chemical termini released by the breakage of nucleotide chains in irradiated DNA.

The procedure involves the proper use of three purified enzymes for labeling the various end-groups and of the method for the exact counting of DNA breaks (see last year Report). When applied under controlled conditions, the technique allows to measure the number of -OH, $-PO_4$ and non functional termini present at the 5' and 3' sides of the internucleotide scissions produced in DNA by ionizing rays.

It was found that mild X-irradiation of isolated mammalian DNA in vitro caused the formation of chain scissions (breaks or gaps) with the

chemical end-groups distributed at the opposing sides according to the following percentages:
68.5 of $-PO_4$, 1.9 of -OH and 29.6 of non-functional unknown termini at the 5' sides,
35.0 of $-PO_4$, 9.5 of -OH and 55.5 of non-functional unknown termini at the 3'sides.

-Irradiated DNA and mammalian nucleic acid polymerases

The nuclear DNA polymerase isolated in our laboratory (see last year Report) and the similar 3.39 S DNA polymerase purified also from cell nuclei by Chang and Bollum were tested for their ability to use irradiated DNA as a primer. Mildly X-irradiated DNA primed the DNA polymerization catalized by the two enzymes at normal initial rates. The finding is in agreement with what was observed few years ago with the cytoplasmic 6-8 S DNA polymerase from calf thymus. The DNA irradiation was found to increase 3 to 4 times the number of DNA ends available in the original sample used for starting the enzymatic polymerization of nucleotides. Obviously the new DNA ends originated by the breakage of polydeoxynucleotide chains following irradiation did not function as effective binding sites for the mammalian DNA polymerases to graft DNA synthesis.

The RNA polymerases A and B were able to start the polymerization of nucleotides at normal rates, when DNA previously exposed to low doses of X-rays was used as a template. These enzymes were rather unspecific in their template requirement for proficient catalysis. In fact, they could transcribed synthetic homopolydeoxynucleotides lacking the full-complement of DNA bases. Otherwise, the mammalian RNA polymerases A and B were very sensitive to the inhibitory action of antibiotics, such a lipiarmycin and a number of rifampicin derivatives, which had chemical affinity for nucleic acid polymerases in general.

- Repairing enzymes

A new preparation of DNA ligase from calf thymus glands was obtained in purified form and without any detectable contamination by DNAses. The enzyme was again tested on irradiated DNA and synthetic poly$(dA)_n$. poly$(dT)_n$ -cellulose substrates with defined radiation damage. Both substrates for DNA ligase were precisely characterized for the chemi-

cal structures present at the nucleotide chain breaks to be sealed by the ligase. It was definitely confirmed the validity of the results previously reportedas indicative data: the enzyme repaired a significant portion of the DNA breaks carrying terminal groups different from the 5'-PO_4//3'-OH functional pair. The mammalian DNA ligase does not appear to be a very selective enzyme in the processes of DNA repair.

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Results of Project No. : 3

Head of Project and scientific staff : J.Booz, M.Coppola, R.Eickel*, H.Menzel*, A.Waker*

Title of Project : Radiation structure of ionizing radiations in tissue and its relation to their spectral energy deposition in biological structures and to the biological effectiveness.

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a) Radiation structure in biological material and in model substances.

The investigation on the behaviour of low energy electrons in tissue equivalent and other gases - W-values, ranges, transmission functions, mass stopping powers, multiple scattering phenomena - was continued. In 1974 the studies were concentrated on the average energy per ion pair, W, of electrons of 17 eV to 5 keV. Results were obtained for tissue equivalent gas (Fig. 1) and methane. For both gases the value of W was found to be constant for electrons of 5 keV and above, and precisely 27.2 eV for methane and 29.4 eV for tissue equivalent gas. Measurements with other gases are in preparation with the aim to get more information on the so far unexplained bump between 0.2 and 1 keV.

The measurements of the distance restricted linear energy transfer, LET_r, and its variance for protons and deuterons of 0.5 to 2 MeV have been terminated. Fig. 2 gives preliminary results of LET_r/LET_∞ of protons as a function of the radial distance r.

Experimental studies on the mass stopping power and the differential W-value of low energy ions have been initiated.

b) Evaluation of the energy deposition of different types of radiations to small biological volumes and its relation to the corresponding biological effectiveness.

Extensive experimental studies on the spectral energy deposition of low LET-radiations - X-rays of 30, 60, 100 and 200 KV and gamma-rays of Cs-137 and Co-60 - have been performed. For the simulation of the sensitive volumes, spherical and cylindrical walled and wall-less counters have been used. Thus it was possible to study the influence of radiation quality, site

* Post-graduate students

diameter (0.1 to 3 μm), counter shape, and wall-effect on the energy deposition spectra. Fig. 3 shows as an example the energy deposition spectra of a cylindrical walled counter simulating a mean volume thickness of 0.65 μm. The curves are normalized to unit event probability. Therefore the areas under the curves represent the mean energy deposition per event for the various radiations.

Experimental and theoretical studies on the spectral energy deposition by fast neutrons to small biological sensitive volumes were carried out. Investigation on the influence of neutron scattering in the wall of a spherical proportional counter on the energy deposition spectra and on the mean lineal energies $\overline{y_F}$ and $\overline{y_D}$ is now completed, apart from the problem of flux attenuation. The neutron scattering acts not only as a scaling factor, i.e. varying the total probability of event observation, but also as a shape modifying factor. This can be seen in Fig. 4, showing the complete energy deposition spectra with and without scattered neutron contribution, for a primary neutron energy of 1.02 MeV.

A project for the experimental determination of fast neutron energy deposition spectra in the low energy range, say from 100 keV to 1 MeV, has been initiated. This study is mostly devoted to the investigation of the high background observed experimentally in the lower energy region of the measured spectra, which is supposed to be due to gamma-ray contamination of the neutron flux.

The same problem, seen from the dosimetric viewpoint, is being studied in the framework of a project of fast neutron dosimetry in a mixed field. Standard neutron sensitive and insensitive ionization chambers and laboratory designed chambers are used for this study.

On the basis of earlier experimental results theoretical studies on the variance of the energy deposition by fast ions in thin layers and in small spheres of biological tissue have been performed, and the relation of the variance to the observed radiobiological effectiveness has been examined. The scope of this study is to see whether the available biological and physical data requires the assumption of a biological threshold.

Local dose distributions inside and outside the follicles of the human thyroid glandcontaminated with I-125 have been calculated.

Experiments on neutron spectrometry and neutron flux measurements using a ^{3}He-spectrometer, a proton recoil gas telescope, and a variable geometry long-counter are being continued.

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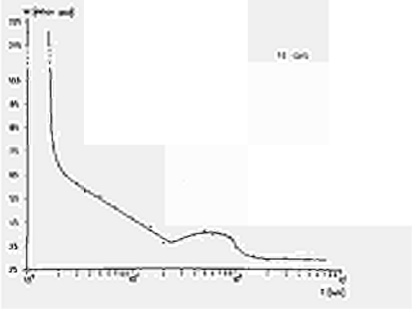

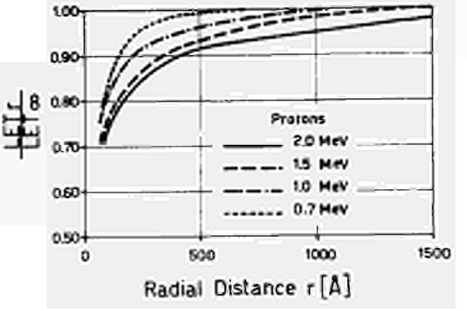

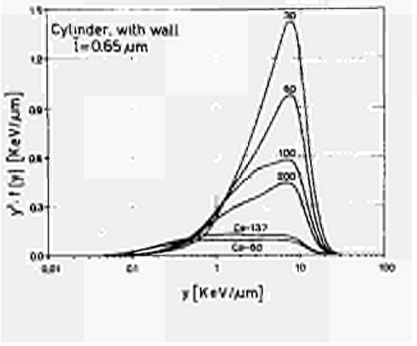

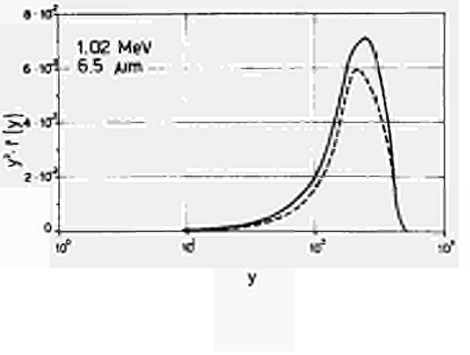

Fig. 1 : W-value of electrons in tissue equivalent gas.

Fig. 2 : LET_r / LET_∞ of protons as a function of the radial distance r.

Fig. 3 : $y^2.f(y)$ as a function of y for various low LET-radiations. Equal areas under the curves represent equal energy contributions.

Fig. 4 : $y^2.f(y)$ for fast neutrons of 1.02 MeV. The solid line represents the energy deposition spectrum including the contribution from scattered neutrons, the dashed line gives the spectrum of the energy deposition from first neutron collisions only.

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