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EURATOM

Årsberetning 1972

PROGRAM BIOLOGI - SUNDHEDSBESKYTTELSE

Jahresbericht 1972

PROGRAMM BIOLOGIE - GESUNDHEITSSCHUTZ

Annual Report 1972

PROGRAMME BIOLOGY - HEALTH PROTECTION

Rapport Annuel 1972

PROGRAMME BIOLOGIE - PROTECTION SANITAIRE

Relazione Annuale 1972

PROGRAMMA BIOLOGIA - PROTEZIONE SANITARIA

Jaarverslag 1972

PROGRAMMA BIOLOGIE - GEZONDHEIDSBESCHERMING

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Fordelingskoden er tilpasset biologi-afdelingens forskellige arbejdsområder. De rubrikker, der svarer til Deres interessefelter, bedes forsynet med et X.

<input type="checkbox"/> 1. Radioaktiv miljøforurening.	<input type="checkbox"/> 5. Strålingsmåling og dens fortolkning; dosimetri.
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<input type="checkbox"/> 3. Strålingsvirkninger på kort sigt, akut strålingssyndrom og dets behandling.	<input type="checkbox"/> 7. Anvendelse af strålingsbeskyttelsens, strålingsbiologiens og kernteknikkens resultater inden for landbrugsforskning.
<input type="checkbox"/> 4. Strålingsvirkninger på langt sigt og inkorporerede radionukleiders toksikologi.	

Biologi afdelingen under Kommissionen for De europæiske Fællesskaber tilsender Dem herved afdelingens almindelige beretning for 1972. Såfremt De er interesseret i at blive optaget på vor forsendelsesliste bedes De tilbagesende dette kort i udfyldt stand (maskinskrivet). ↑

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<input type="checkbox"/> 1. Radioaktive Kontamination der Umwelt.	<input type="checkbox"/> 5. Strahlenmessung und ihre Interpretation, Dosimetrie.
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<input type="checkbox"/> 4. Spätwirkungen bei Bestrahlung und Toxikologie inkorporierter Radionuklide.	

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200, rue de la Loi
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<input type="checkbox"/> 1. Radioactive contamination of the environment.	<input type="checkbox"/> 5. Measurement of radiation and its interpretation, dosimetry.
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<input type="checkbox"/> 4. Long-term effects of radiation and toxicology of ingested radionuclides.	

The Biology Division of the Commission of the European Communities sends you herewith its Annual Report for 1972. If you would like to be put on our mailing list, please return us this card duly completed (typewritten).

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<input type="checkbox"/> 3. Effets à court terme des rayonnements, syndrome aigu d'irradiation et son traitement.	<input type="checkbox"/> 7. Application des connaissances acquises en radioprotection, radiobiologie et techniques nucléaires à la recherche agronomique.
<input type="checkbox"/> 4. Effets à long terme des rayonnements et toxicologie des radionuclides ingérés.	

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<input type="checkbox"/> 2. Effetti ereditari delle radiazioni.	<input type="checkbox"/> 6. Applicazione alla ricerca medica delle conoscenze acquisite in radioprotezione, radiobiologia e tecniche nucleari.
<input type="checkbox"/> 3. Effetti a breve termine delle radiazioni, sindrome acuta da irradiazione e suo trattamento.	<input type="checkbox"/> 7. Applicazione alla ricerca agronomica delle conoscenze acquisite in radioprotezione, radiobiologia e tecniche nucleari.
<input type="checkbox"/> 4. Effetti a lungo termine delle radiazioni e tossicologia dei radionuclidi incorporati.	

Le inviamo, qui acclusa, la Relazione annuale 1972 dei Servizi di Biologia della Commissione delle Comunità europee. Se desidera che il Suo nome figuri fra i destinatari delle nostre pubblicazioni, La preghiamo di restituirci il presente modulo debitamente compilato (a macchina).



De afdeling Biologie van de Commissie van de Europese Gemeenschappen zendt U hierbij haar Jaarverslag 1972. Indien U wenst in onze verzendlijst te worden opgenomen, gelieve ons deze kaart volledig ingevuld te retourneren (machineschrift).



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<input type="checkbox"/> 2. Genetische stralingseffecten.	<input type="checkbox"/> 6. Toepassing van de verworven kennis op het gebied van stralingsbescherming, stralingsbiologie en kerntechniek bij medisch onderzoek.
<input type="checkbox"/> 3. Effecten van straling op korte termijn, acuut bestralingssyndroom en behandeling.	<input type="checkbox"/> 7. Toepassing van de verworven kennis op het gebied van stralingsbescherming, stralingsbiologie en kerntechniek bij landbouwkundig onderzoek.
<input type="checkbox"/> 4. Effecten van straling op langer termijn en toxicologie van opgenomen radionucliden.	

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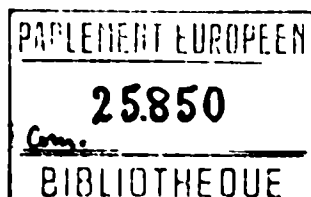
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* Der Jahresbericht 1972 war noch nicht verfügbar.
The annual report 1972 was not yet available.
Le rapport annuel 1972 n'était pas encore disponible.

Der Ministerrat der Europäischen Gemeinschaften traf erst im Juni 1971 seine Entscheidung über das neue Mehrjahresprogramm "Biologie - Gesundheitsschutz". Am 1. Januar 1973 traten drei weitere Mitgliedstaaten den Gemeinschaften bei. Daraus ergab sich, daß 1972 das erste und einzige vollständige Arbeitsjahr des neuen Programms "zu Sechs" war.

Dieses Jahr wurde zu einem großen Teil für Vertragsverhandlungen sowie Neuformulierungen und Verbesserungen der Verträge genutzt, von denen die meisten inzwischen unterzeichnet und in Kraft getreten sind.

Außerdem fanden zahlreiche orientierende Diskussionen und Besuche zur Vorbereitung des Beitritts der neuen Mitglieder statt, und ein Vorschlag über die damit verbundene Anpassung der Programme wurde dem Ministerrat im November 1972 vorgelegt. Wir möchten an dieser Stelle unseren Kollegen in den neuen Mitgliedsländern und auch in Norwegen unseren Dank aussprechen für die freundliche Aufnahme, die sie uns bereiteten und für die konstruktiven Gespräche über eine Vielfalt von technischen, politischen und Verwaltungsproblemen. Diese Entwicklung gipfelte in der ersten Teilnahme der Delegationen der neuen Mitgliedsländer als Beobachter an der Dezembersitzung des Beratenden Programmausschusses "Biologie - Gesundheitsschutz".

Viele Überlegungen galten ebenfalls während dieses Jahres den Verwaltungsmethoden des Programms und seiner Forschungsprojekte mit dem Ergebnis, daß einige neue administrative Vereinfachungen einge-

führt und auch bereits auf die 1972 unterzeichneten Mehrjahresverträge angewandt wurden. Unser besonderer Dank gilt den Mitgliedern des Beratenden Programmausschusses, die uns mit wertvollem Rat zur Seite standen.

Vor diesem Hintergrund machte die Forschung gute Fortschritte. Der Leser wird auf den folgenden Seiten die Arbeit des Jahres 1977 in den eigenen Worten der verantwortlichen Forscher dargestellt finden.

Es handelt sich um ein weites Gebiet, in dem jeder anregende neue Ergebnisse antreffen wird. Genetiker werden von den letzten Hinweisen auf Dosis-Mutation-Beziehungen bei non-disjunction angesprochen werden. Diejenigen, die sich mit internen Emitttern befassen, werden die Menge der Arbeiten über Ra^{224} -Untersuchungen begrüßen. Diejenigen, die maximal zulässige Dosen detailliert berechnen müssen, aber sich auch über die zugrunde liegenden Mechanismen informieren wollen, werden auf die Ergebnisse der Arbeiten über die Lunge und über aus der Luft inhalierte Radionuklide verwiesen. Biophysiker und Immunologen werden weitere Interessengebiete entdecken. Neue Perspektiven und Konzepte haben sich bei den Primäreffekten und Reparationsvorgängen ergeben. Neue Techniken wurden in der Zytogenetik eingeführt. Die Methoden der Lokalisierung von Ionen in subzellularen Strukturen, für die Dosimetrie auf zellulärer und subzellulärer Ebene (Mikrodosimetrie) und für die Neutronendosimetrie sind weiter vervollkommen worden. Es wäre ungerecht, Namen einzelner zu nennen. Die Kompetenz von allen und ihren großen Einsatz möchten wir hervorheben und ihnen für ihre Arbeit am Programm Biologie - Gesundheitsschutz danken.

Raymond K. APPLEYARD

Fierre RECHT

INTRODUCTION

E

The Council of Ministers of the European Communities decided upon the new pluriannual programme "Biology - Health Protection" only in June 1971. On 1st January 1973, three new Member States joined the Communities. In consequence, 1972 was the first and only full year of operation of the new programme "à Six".

It was therefore spent quite largely in renegotiating and redrafting new and improved contracts, most of which are now signed and in operation.

In addition, many exploratory discussions and visits took place to prepare the entry of the new Members and a plan for the adaptation of the programme to this event was placed before the Council of Ministers in November. We would like to thank our many colleagues in the new Member States as well as in Norway, who received us with great friendliness and talked with us most constructively of the wide range of technical, policy and administrative complexities involved. This process culminated in the welcome presence at the December meeting of our Advisory Committee on Programme Management of observer delegations from the new Member States.

Much reflection was also devoted during the year to the method of management of the programme and its projects, with the result that some new administrative procedures were brought into play, which were applied to the pluriannual contracts signed in 1972. We would like to thank particularly the members of our Advisory Committee on Programme Management "Biology - Health Protection" who helped with their advice.

Against this background, the research went solidly on. The reader will find the year's work presented in the following pages by those who were responsible for it in their own words.

The field is a wide one and each will find progress that excites him. Geneticists will be interested by the progress concerning the dose mutation relation for non-disjunction. Those responsible for problems of internal emitters will note the solid stream of papers now emerging from the Ra²²⁴ work. Those who must compute maximum levels in detail but would like to know about the mechanisms will note the emergence of work on the lung and air-borne entry. Biophysicists and immunologists will find other interests. New data and concepts are emerging in the field of primary effects and repair processes. New techniques are introduced in cytogenetics. Methods for localising ions in subcellular structures, for the dosimetry at the cellular and subcellular level (microdosimetry) and for neutron dosimetry have been improved. It would be invidious to mention names. To all, we would like to express our pleasure in their technical competence and hard work and our thanks for their cooperation.

Raymond K. APPLEYARD

Pierre RECHT

INTRODUCTION

F

Le Conseil de Ministres des Communautés européennes s'est prononcé sur le nouveau programme pluriannuel "Biologie - Protection Sanitaire" en juin 1971 seulement. A la date du 1er janvier 1973, trois nouveaux Etats membres se sont joints aux Communautés. En conséquence, l'année 1972 a été la première et la seule année complète de fonctionnement du nouveau programme à Six.

C'est la raison pour laquelle 1972 fut en majeure partie consacrée à renégocier et rédiger de nouveaux contrats améliorés, dont la plupart sont maintenant signés et mis en oeuvre.

De plus, de nombreuses discussions et visites exploratoires ont eu lieu afin de préparer l'entrée des nouveaux membres, et un plan d'adaptation du programme a été soumis, à cet effet, au Conseil de Ministres en novembre. Nous souhaiterions adresser aux nombreux collègues, qui dans les nouveaux Etats membres aussi bien qu'en Norvège nous ont reçu avec beaucoup d'amitié, et ont eu avec nous les échanges les plus constructifs dans un vaste éventail de problèmes de technique, d'administration et de doctrine. Ce processus s'est soldé, en 1972, par la présence appréciée des délégations d'observateurs des nouveaux Etats membres à la réunion de décembre de notre Comité Consultatif en matière de gestion de programmes.

On s'est beaucoup penché aussi au cours de cette année, sur la méthode de la gestion du programme et de ses projets, avec pour conséquence l'adoption de quelques nouvelles procédures administratives, appliquées dans les contrats pluriannuels signés en 1972. Nous voudrions remercier tout particulièrement les membres de notre Comité Consultatif en matière de gestion de programmes "Biologie -

Protection Sanitaire" qui nous ont aidé de leur expérience.

Vu dans ce contexte, la recherche a progressé sérieusement. Le lecteur trouvera le travail de l'année présenté dans les pages suivantes par ceux qui en étaient responsables dans les termes choisis par eux.

Le domaine est vaste, et chacun y trouvera matière à intérêt. Les généticiens s'arrêteront sur les progrès relatifs à la relation dose - mutation pour non-disjonction. Les responsables des problèmes des émetteurs internes noteront l'impressionnant afflux de publications provenant maintenant du travail sur le Ra²²⁴. Ceux qui doivent analyser les niveaux maximum en détail, mais qui aimeraient être informés des mécanismes seront intéressés par le travail sur la voie d'entrée atmosphérique et pulmonaire. Les biophysiciens et les immunologistes trouveront d'autres sources d'intérêt. Des données et des concepts nouveaux se révèlent dans les domaines des effets primaires et des processus de réparation. Des techniques nouvelles sont introduites en cytogénétique. Des méthodes pour localisation d'ions dans les structures subcellulaires, pour la dosimétrie au niveau cellulaire et subcellulaire (micro-dosimétrie), et pour la dosimétrie des neutrons ont été améliorées. Il serait hors de propos, dans cette courte introduction, de citer des noms. Mais que tous ceux qui ont participé à notre programme soient remerciés pour leur coopération et sachent que leur compétence technique et l'effort qu'ils ont fourni sont hautement appréciés.

Raymond K. APPLEYARD

Pierre RECHT

INTRODUZIONE

Soltanto nel giugno 1971 il Consiglio dei Ministri delle Comunità europee ha adottato il nuovo programma pluriennale "Biologia e protezione sanitaria". Il 1° gennaio del 1973 tre nuovi Stati membri hanno aderito alla Comunità. Si può dire, quindi, che il 1972 è stato il primo ed unico anno di piena attuazione del nuovo programma "a sei".

Pertanto l'anno è stato impiegato per nuovi negoziati e per rifare delle stesure di contratti nuovi e perfezionati, molti dei quali sono già stati firmati e sono operanti.

Inoltre sono state intavolate molte discussioni a carattere esplorativo e sono state fatte delle visite per preparare l'entrata dei nuovi Stati membri; a tale scopo in novembre, è stato presentato al Consiglio dei Ministri, un progetto di adattamento del programma. Vorremmo ringraziare i numerosi colleghi dei nuovi Stati membri, nonché i colleghi norvegesi, che ci hanno accolti con grande cordialità intrattenendosi con noi molto costruttivamente sulla vasta gamma dei problemi tecnici, politici e amministrativi. Queste attività sono culminate con la gradita presenza alla riunione di dicembre del nostro Comitato consultivo in materia di gestione dei programmi di delegazioni di osservatori dei nuovi Stati membri.

Nel corso dell'anno si è prestata molta attenzione al metodo di gestione del programma e ai suoi progetti, con il risultato che sono entrate in gioco alcune nuove procedure amministrative da applicarsi ai contratti pluriennali firmati nel 1972. Ringraziamo, in particolare, i membri del nostro Comitato consultivo in materia di gestione dei programmi "Biologia e protezione sanitaria" i cui pareri ci sono stati di grande aiuto.

Su queste basi, la ricerca è stata sempre più intensificata. Il lettore troverà i lavori eseguiti durante l'anno, presentati nelle pagine seguenti dagli autori stessi.

Il campo è vasto e ognuno vi troverà l'argomento che lo interessa. I genetisti saranno interessati dal progresso in materia di rapporto dose/mutazione per la non disgiunzione. I responsabili dei problemi degli emettitori interni noteranno l'abbondante produzione di documenti che hanno avuto origine dal lavoro sul RA 224. Coloro che devono calcolare dettagliatamente i livelli massimi, ma che gradirebbero conoscere qualcosa circa i meccanismi noteranno l'apparizione di lavori sui polmoni e sull'assimilazione attraverso l'aria. I biofisici e gli immunologi troveranno altri soggetti interessanti. Nuovi dati e concetti emergono nel campo degli effetti primari e nei processi di rigenerazione. Nuove tecniche vengono introdotte nella citogenetica. Sono stati perfezionati i metodi per la localizzazione degli ioni nelle strutture subcellulari, e sono state migliorate la dosimetria a livello cellulare e subcellulare (microdosimetria) e la dosimetria dei neutroni. Non è il caso di far nomi. A tutti vorremmo esprimere il nostro apprezzamento per la loro competenza tecnica e il duro lavoro ed i nostri ringraziamenti per la loro collaborazione.

Raymond K. APPLEBYARD

Pierre RECHT

INLEIDING

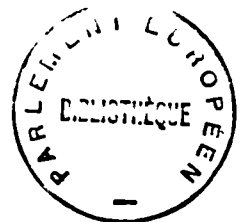
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De Raad van Ministers van de Europese Gemeenschappen heeft pas in juni 1971 een besluit genomen met betrekking tot het nieuwe meerjarenprogramma "Biologie - Bescherming van de gezondheid". Voorts zijn op 1 januari 1973 drie nieuwe staten tot de Gemeenschap toegetroeden. Een en ander heeft tot gevolg gehad dat 1972 het eerste en enige volle jaar is geweest, waarin het nieuwe programma "van de Zes" van toepassing was.

Dit jaar stond daardoor grotendeels in het teken van nieuwe onderhandelingen met het oog op nieuwe, herziene contracten waarvan de meeste thans ondertekend en van kracht zijn.

Daarnaast werden talloze verkennende besprekingen gevoerd, respectievelijk verkennende bezoeken afgelegd om de toetreding van de nieuwe leden voor te bereiden; in november werd aan de Raad van Ministers een ontwerp voorgelegd tot aanpassing van het programma aan de nieuwe situatie. Wij willen hierbij onze vele collega's in de nieuwe lid-staten en in Noorwegen danken die ons zeer hartelijk hebben ontvangen en die ons in zeer constructieve zin hebben onderhouden over de talrijke technische, politieke en administratieve problemen waarmee men in dit verband wordt geconfronteerd. De zeer gewaardeerde aanwezigheid van de door de nieuwe lid-staten gedelegeerde waarnemers in de decembervergadering van ons raadgevend comité voor programmabeheer vormde in dit opzicht een hoogtepunt.

In 1972 werd ook ernstig nagedacht over de methode die bij het beheer van het programma en de daarin opgenomen projecten moet worden toegepast. Dit had tot resultaat dat enkele nieuwe administratieve procedures werden vastgesteld en vervolgens toegepast op de in 1972 ondertekende meerjarencontracten. Onze dank gaat hierbij in het bijzonder naar de leden van ons raadgevend comité voor programmabeheer die ons met hun adviezen hebben bijgestaan.



Tegen deze achtergrond werden de onderzoekswerkzaamheden ijverig voortgezet. In de hierna volgende bladzijden brengen degenen die in 1972 voor deze werkzaamheden verantwoordelijk waren in hun eigen bewoordingen daarover verslag uit.

Het daarbij bestreken terrein is zeer uitgestrekt, zodat iedere lezer wel een of andere vooruitgang zal ontdekken die hem zal interesseren. Genetici zullen met de grootste belangstelling kennis nemen van de vooruitgang die op het gebied van de "non-disjunction" dosis/mutatieverhouding werd geboekt. Wie vraagstukken in verband met de interne stralers bestudeert, zal de stroom van documenten noteren die de werkzaamheden aan Ra²²⁴ thans opleveren. Wie maximale niveaus en detail moet bepalen en alles wil afweten van de mechanismen zal met voldoening constateren dat nu ook werkzaamheden met betrekking tot de longen en uit de lucht geïnhaleerde radionucliden worden gerealiseerd. Biofysici en immunologen zullen er eveneens wat van hun gading vinden. Op het gebied van de primaire effecten en herstelprocessen verschijnen nieuwe gegevens en begrippen. Nieuwe technieken worden toegepast in de cytogenetica. Voorts werden de methoden voor het lokaliseren van ionen in subcellulaire structuren, voor dosismeting op cellulair en subcellulair niveau (microdosimetrie) en voor neutronendosimetrie verbeterd. Namen kunnen hier moeilijk worden genoemd. Allen die door hun uitgebreide kennis en harde werken tot die resultaten hebben bijgedragen, betuigen wij hierbij onze hartelijke dank.

Raymond K. APPLEYARD

Pierre RECHT

Mitglieder im Jahr 1972 des Beratenden Programmausschusses
"BIOLOGIE - GESUNDHEITSSCHUTZ"

Members in 1972 of the Advisory Committee on Programme Management
"BIOLOGY - HEALTH PROTECTION"

Membres en 1972 du Comité Consultatif en matière de gestion de programmes
"BIOLOGIE - PROTECTION SANITAIRE"

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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

Group Contract
on
D O S I M E T R Y
in Biology, Medicine and Agriculture

1. Research on dosimetry although generally rather dispersed, is an indispensable activity within radiobiological or other radiation research institutes. Therefore it seemed necessary to initiate close cooperation between a number of dosimetry - research units in the Communities. The intention was

- to bring together through collaborative programmes the dispersed efforts
- to provide an adequate structure for approaching difficult dosimetry problems in common efforts (e.g. microdosimetry, neutron dosimetry, intercomparison programmes) and
- to provide effective services for other projects in the Programme "Biology- Health Protection" of the Commission (e.g. EULEP Committee for dosimetry standardisation).

These three objectives have been achieved in varying degrees, and continuing work is necessary. One of the achievements has been the formation of a Dosimetry Group initiated by the Commission together with scientists from various institutions within the European Communities actively working on dosimetry.

2. The following research units are members of the Dosimetry Group and are participating in a collaborative research programme:

- Radiobiological Institute TNO, Rijswijk¹⁾
- C.P.A., Université Paul Sabatier, Toulouse¹⁾
- GSF, Abtlg. für Biophysikalische Strahlenforschung, Frankfurt¹⁾

- GSF, Institut für Strahlenschutz, Neuherberg/München¹⁾
- L.B.R.M., Université Louis Pasteur, Strasbourg¹⁾
- Institut für Biophysik, Universität des Saarlandes, Homburg¹⁾
- CNEN, Lab. Dosimetria e Standardizzazione, Casaccia²⁾
- Institute of the Association EURATOM-ITAL, Wageningen³⁾
- Biology Group, CEC, Ispra⁴⁾

3. The general aim of the collaborative programme of the Dosimetry Group is research on dosimetry for biology, medicine and agriculture and the practical use of this knowledge. It serves as physical basis for scientific work in these fields. The Dosimetry Group has concentrated its collaborative efforts on three major subjects, essential for understanding of the mechanisms of radiation effects and for estimating the radiation risk:

- evaluation of the biological effectiveness of different types of radiation
- energy transfer in biological material and model substances
- radiation effects in condensed matter, its application and development of new dosimetric methods.

The results of the work for 1972 are outlined on the following pages by different members of the Dosimetry Group.

4. The group has held half-yearly meetings which have actively influenced the collaboration between the members and so achieved

1) Contract nr. 101-72-1 BIOC

2) Contract nr. 068-67-6 BIOD

3) The report on the activities in dosimetry is incorporated in the annual report 1972 of contract nr. 094-72-1 BIAN

4) The report on the activities in dosimetry is incorporated in the annual report 1972 of the Biology Group, Ispra.

success and progress of the work. Additional possibilities for mutual exchange of information and a guarantee of high standard of work from the members of the group are the Symposia on Microdosimetry which are held every two years (proceedings of the last symposium were published in February 1972 as Euratom report nr. EUR 4810) and the Symposium on Neutron Dosimetry in Biology and Medicine held on May 15-19, 1972 (proceedings published in September 1972 as Euratom report nr. EUR 4896).

5. Scientific correlation exists between the work of the Dosimetry Group and another group contract on neutron dosimetry and inter-comparison (contract nr. 113-72-1 BIOC); the results of which are given later in the present report. Since the research programmes are complementary, and several institutes are collaborating in both of them, the exchange of information is assured at each level.

Contract No. 101-72-1 BIOC

Radiobiological Institute TNO, Rijswijk (ZH), The Netherlands

G.W. Barendsen

Evaluation of the biological effectiveness of different types of radiation

The aim of these studies is the correlation of the pattern of energy deposition by various types of radiation and the effectiveness of these radiations per unit absorbed energy for producing biological damage. So far, experiments have been carried out to determine frequency distributions of locally absorbed energy for α -particles and protons of different energies. The data for α -particles have been correlated with their effectiveness for induction of damage to the clonogenic capacity of cultured cells. This correlation was based on a hypothesis assuming that this damage is initiated through the action of a single particle which deposits in a critical structure an amount of energy exceeding a specified threshold.

The data for protons were obtained as a basis for the interpretation of results to be derived with fast neutrons.

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

Evaluation of the biological effectiveness of different types of radiation

In order to relate cross section data for impairment to the reproductive capacity for cultured cells of human kidney origin irradiated with different types of radiation to microdosimetric quantities, event size distributions for α -particles from ^{210}Po and for protons of energies of 1.5 and 3.0 MeV have been measured with a tissue-equivalent proportional counter. Simulated diameters for critical structures ranging from 5.6 μm to 0.075 μm were obtained by variation of the pressure of tissue-equivalent gas.

After passage of two Mylar absorbers of 0.54 mg/cm^2 each, the particles enter the sensitive volume of the proportional counter through a circular aperture in the wall of the counter. The axis of the aperture is perpendicular to the central wire. The Mylar windows are required to separate the sensitive volume and the particle source from outside air. The energy of the particles was varied by inserting additional Mylar absorbers in the particle beam.

Energy loss distributions for α -particles of different energies have been measured at equivalent diameters of 5.6, 1.2, 0.63, 0.15 and 0.075 μm of unit density tissue respectively. Measured energy loss distributions for α -particles which have passed through different numbers of absorbers for a simulated diameter of 0.15 μm are presented in figure 1. The mean values of the energy loss distributions for simulated diameters of 1.2 μm and 0.075 μm have been fitted to the LET_{∞} versus range curve for α -particles, derived from data published by Walsh (1970). The results of this analysis are presented in figure 2. From this curve the LET_{∞} of the α -particles which have passed through 2, 3, 4, 5 and 6 absorbers were determined to be equal to 110, 126, 148, 200 and 215 $\text{keV}/\mu\text{m}$ respectively.

At simulated critical structure diameters of 0.15 and 0.3 μm , energy loss distributions have also been measured for protons accelerated to initial energies of 1.5 MeV and 3.0 MeV. The results for protons of 1.5 MeV at a simulated dia-

meter of 0.15 μm are given in figure 3.

An identical analysis as performed for the energy deposition distributions of the α -particles has been carried out for the local energy distributions of the protons. The result is presented in figure 4 and compared with an LET_{∞} versus range curve obtained from data published by Oldenburg and Booz (1972).

The energy distributions for α -particles as well as for protons have further been compared with Vavilov distributions. Distributions obtained with the α -particles were in fair agreement with the theoretical distributions. For protons, however, there was a large discrepancy between measured and theoretical distributions which might be due to spread in initial energy of the accelerated particles. Since the interpretation of the biological effectiveness of fast neutrons depends in part on the knowledge of the energy deposition characteristics of protons, this will be a subject for further investigations.

References

- Oldenburg, U., and Booz, J., Euratom report EUR 4786 I, e (1972).
Walsh, P.J., *Health Phys.* 19 (1970) 312.

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

- Hogeweg, B. and Barendsen, G.W., Local energy distributions for α -particles of different energies in relation to the evaluation of critical sizes and energy requirements involved in the induction of damage in mammalian cells. In: *Proc. Third Symp. on Microdosimetry, Stresa, 1971. Commission of the European Communities, Euratom, Luxembourg, pp. 857-872 (1972).*
Barendsen, G.W., Concluding remarks biological aspects. *Idem*, pp. 905-913.

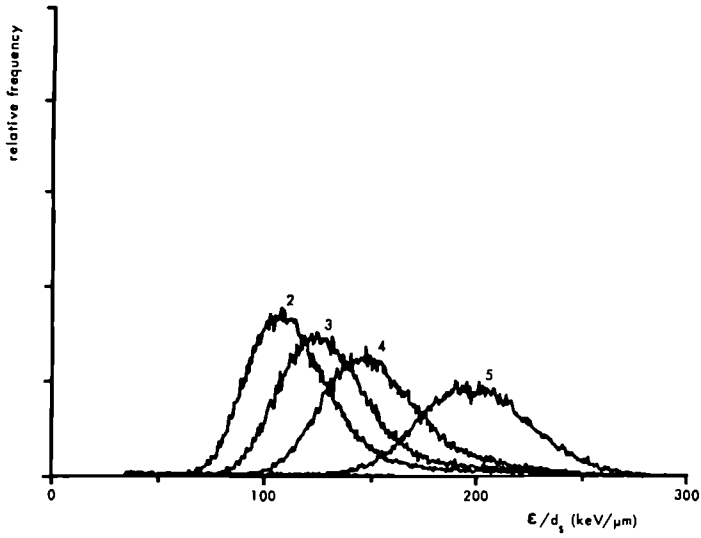


Figure 1. Energy loss (ϵ) distributions of ^{210}Po α -particles, which have passed through different numbers of absorbers, at simulated diameter (d_s) of $0.15 \mu\text{m}$ tissue density 1. Numbers correspond to number of absorbers.

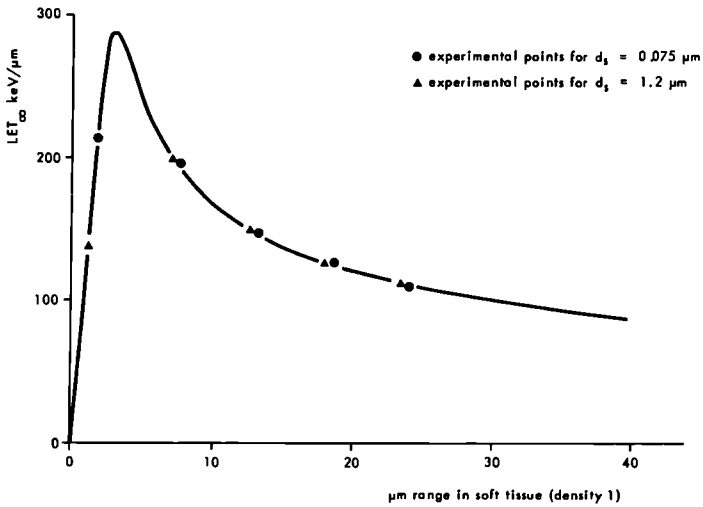


Figure 2. LET_∞ versus range in soft tissue for α -particles. The curve is obtained from data published by Walsh (1970).

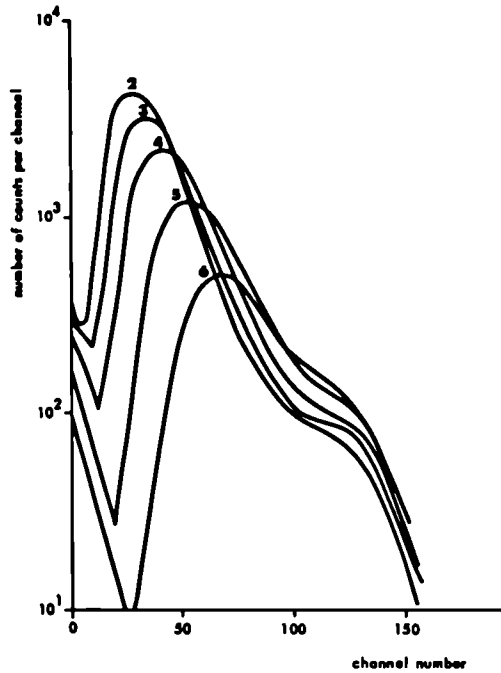


Figure 3. Energy loss distributions of 1.5 MeV protons, after passage through different numbers of absorbers. Numbers correspond to number of absorbers.

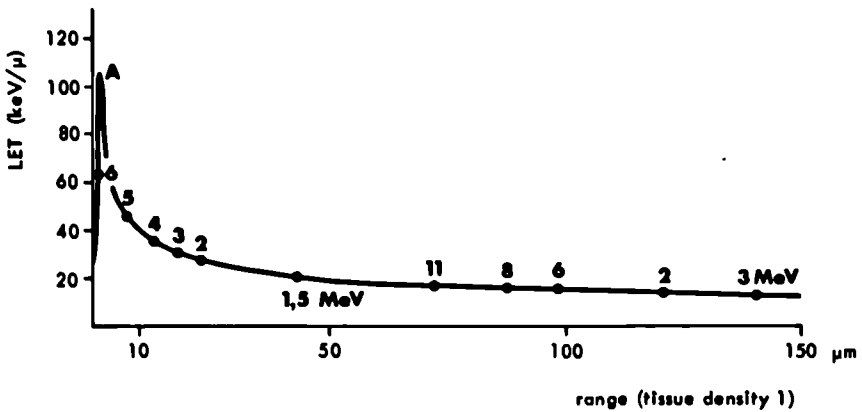


Figure 4. LET_{∞} versus range for protons. Experimental points are for 1.5 MeV protons which have passed through 2, 3, 4, 5 and 6 absorbers respectively and are for 3 MeV protons after passage through 2, 6, 8 and 11 absorbers respectively. Curve A is obtained from data published by Oldenburg and Booz (1972).

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.

Nous avons terminé l'étude complète du ralentissement des photons et des électrons d'énergie supérieure à 2 MeV par l'introduction et la simulation du Bremsstrahlung et nous avons pu l'appliquer à divers problèmes.

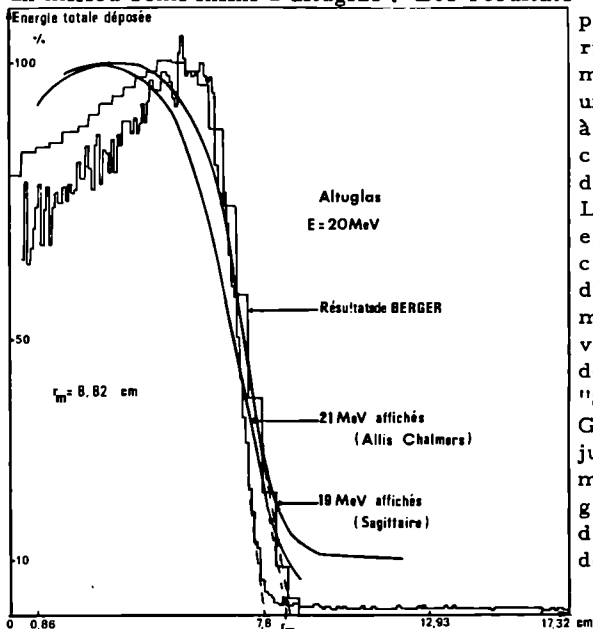
Dans le domaine des basses énergies, nous calculons les sections efficaces élastiques et inélastiques ce qui est fondamental pour la suite des recherches.

Résultats du projet n° 1

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

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

La simulation du rayonnement de freinage des électrons d'énergie supérieure à 2 MeV est réalisée par échantillonnage de la formule de SCHIFF (1). Nous appliquons ainsi nos programmes à l'étude de la pénétration d'électrons de 30, 20 ou 10 MeV (énergies rencontrées en radiothérapie) pour obtenir toutes les quantités dosimétriques et microdosimétriques telles que répartition spatiale de l'énergie, spectres de particules à différentes profondeurs, spectres de TLE et TLE moyen. Nous donnons ici par exemple l'énergie totale déposée en profondeur par un faisceau d'électrons monoénergétiques de 20 MeV tombant perpendiculairement sur un milieu semi infini d'altuglas. Les résultats de nos calculs sont comparés aux résultats expérimentaux obtenus par des mesures d'ionisation avec une chambre d'ionisation à remplissage liquide placée à diverses profondeurs dans un fantôme d'altuglas. Les expériences ont été effectuées dans des faisceaux d'électrons issus du béta-tron "Allis Chalmers" des cliniques universitaires de Louvain et de l'accélérateur linéaire "Sagittaire" de l'Institut Gustave Roussy de Villejuif. Nous donnons également les résultats de Berger (M. J.) (2) obtenus pour de l'eau avec des électrons de 20 MeV.



Pour l'étude du transport des électrons d'énergie inférieure à 1000 eV, nous avons commencé les recherches et les calculs de sections efficaces. Dans ce domaine d'énergie - 1000 eV à environ 10 eV - nous devons tenir compte des diffusions élastiques et des diffusions inélastiques (ionisations et excitations). Les autres interactions (phonons, vibrations, etc. . .) . pouvant être négligées.

Les sections efficaces élastiques sont obtenues théoriquement par la formule de Mott (n° 1A 110 dans la nomenclature de Motz (J. W.) (3)).

$$\frac{d\sigma}{d\Omega} = \left(\frac{137r_0}{2p_1} \right)^2 \left| \sum_{l=0}^{\infty} (2l+1) \exp(2i\eta_l - 1) P_l(\cos \theta) \right|^2$$

pour le calcul des phases η_l nous avons besoin du potentiel de l'atome et nous utilisons soit un potentiel de Thomas-Fermi, soit un potentiel exponentiel. La convergence de cette série de polynômes de Legendre est améliorée au moyen de la transformation récurrentielle de Yennie-Ravenhall et Wilson (4). Ainsi, nous pouvons obtenir les distributions angulaires de diffusion élastique pour chaque énergie de l'électron.

Les sections efficaces inélastiques sont pratiquement impossibles à obtenir par le calcul (excepté peut être pour les atomes hydrogénéoïdes) et nous avons été amenés à exploiter des résultats expérimentaux. Pour des corps d'intérêt biologique -H, C, O, N- nous avons rassemblé des sections efficaces inélastiques (par exemple travaux de Fite, Boksenberg, ou Rapp (5), (6), (7)).

Ces résultats expérimentaux sont tabulés, approximés par des fonctions empiriques et nous servent à déterminer pour un électron sur un trajet donné les probabilités de diffusion inélastique.

Références.

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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

The understanding and interpretation of biological effects of ionizing radiation requires information about the local distribution of radiation energy deposition. In project I a special ionizing chamber was developed, by which the absorbed dose can be determined as a function of the distance from the ion track.

The contribution of anorganic and organic radicals to the inactivation of DNA is rather unknown. Since the relevant structures in a cell are too complicated, it is necessary to investigate the inactivating effect of radicals in model systems. In project II an aqueous solution of sulphuric acid with ferrous ions as targets was used to investigate the protective and sensitizing effect of cysteine.

For quantitative analysis of radiobiological investigations in microbiology and for intercomparison of these data appropriate methods must be developed for the determination of absorbed dose in thin layers, the quality of X-rays and the homogeneity of radiation fields. For this purpose in project III the thermoluminescence of thin crystals of CaF_2 were investigated.

Ergebnisse des Projekts Nr. I

Leiter des Projekts und wissenschaftliche
Mitarbeiter: Dr. A Kappos

Titel des Projekts: Determination of the radial
energy distribution of heavy charged particles

For the interpretation of biological effects in the irradiation with heavy charged particles the knowledge of the energy fraction, which is deposited directly in the core of the track, and that fraction, which is deposited indirectly by delta rays in a definite distance from the core, is of great importance. Precise data about radial energy distribution of heavy charged particles are scanty. Theoretical considerations are based on very rough assumptions, for instance rectilinear tracks of delta rays, constant energy loss. Therefore it is necessary to determine the radial energy distribution of such particles experimentally.

The chamber consists of a conducting cylinder (inner diameter: 194 mm, length: 1900 mm) with a electrode fixed excentrically to the ion track (electrode diameter: 2 mm, distance from the chamber axis: 10 mm). The particle beam enters the chamber centrally. For defining the measuring volume the electrode consists of three parts, which are isolated against each other by teflon. The middle part is the collecting electrode with a length of 100 mm; at both ends of the collecting electrode are the screening electrodes. To avoid the backscattering of electrons from the chamber wall a grid of 0.2 mm thick steal wire is installed. The distance between the wires amounts 2.6 mm. The distance between grid and chamber wall amounts 10 mm. The positive voltage is put on the grid. The chamber is filled with gas at a definite pressure. When the pressure is high enough, all delta rays produced by the primary particles are stopped within the measuring volume. Then, on the collecting electrode all the

ions are recorded, which correspond to the energy loss of the primary particles along the collecting electrode. Naturally, this is only the case, when equilibrium is reached between such delta rays, which are produced before entering the measuring volume and deliver their energy within this volume, and those electrons, which are produced in the measuring volume and deliver their energy outside the measuring volume. The conditions for this equilibrium are fulfilled, if firstly the length of the screening electrodes are greater than the range of the most energetic delta rays in the gas and secondly the stoppingpower of the gas for the primary particles is constant during the passage through the chamber.

When the gas pressure is lowered, not all delta rays are stopped totally within the chamber. A part of them reaches the chamber wall and is absorbed there. The ions, which wereproduced by the remaining energy, are not recorded on the measuring electrode. The measured current will be smaller and corresponds to the energy, which is deposited within a cylinder around the beam of the primary particles. The radius of this cylinder is defined by the gas pressure. By variation of the gas pressure in the chamber instead of changing the chamber dimensions the function LET_r can be determined. Differentiation of this function to r yields the energy deposited in a cylinder ring of the radius r and the thickness dr . Condition for this calculation is the knowledge of the W -value. Experimental values for $W(E)$ (Total absorption of an electron with the energy E) and $w(E)$ (For an electron with an energy between E and $E + dE$) are mentioned by COLE (1969) for delta rays between 20 eV and 50 keV in air. These values will be used in determining the radial energy distribution forprotons with energies up to 600 keV.

Reference :

COLE, A.: Rad.Res. 38 (1969), 7

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

For the interpretation of protection respectively sensitization in biological systems it is necessary to investigate these effects in model systems. As a simple model system an aqueous solution of sulphuric acid was used, to which were added ferrous ions as targets and cysteine as the modifying agent. The concentration of the ferrous ions amounted 10^{-3} mol/l in all experiments, the concentration of cysteine varied between zero and 1 mol/l. Aerated and deaerated solutions were irradiated with X-rays up to 40 krad.

One of the experimental results is shown in figure 1. The quotient

$$Q = \frac{c_{\text{Fe}^{3+}}^*}{c_{\text{Fe}^{3+}}^0}$$

is plotted against the cysteine concentration, whereby $c_{\text{Fe}^{3+}}^*$ is the concentration of ferric ions in the ferrous-cysteine solution and $c_{\text{Fe}^{3+}}^0$ the concentration of the ferric ions in the pure ferrous solution. $Q > 1$ means sensitization, $Q < 1$ protection. In aerated solutions at low cysteine concentration sensitization is obtained. With increasing cysteine concentration sensitization increases, reaches a maximum at 2×10^{-3} mol/l and then decreases. Complete protection is achieved at 2×10^{-2} mol/l cysteine.

In contrast to aerated solutions no sensitization can be observed in deaerated solutions. Protection is achieved for cysteine concentrations higher than 10^{-3} mol/l. Complete protection is obtained for cysteine concentrations higher than 5×10^{-2} mol/l.

The interpretation of the experimental results may be followed best regarding the reactionscheme in figure 2. The main radiolyse products in deaerated acid solutions are OH^\bullet - and H^\bullet - radicals, in aerated acid solutions OH^\bullet - and HO_2^\bullet - radicals. In figure 2 the radicals OH^\bullet , H^\bullet and HO_2^\bullet are denoted as A^\bullet . In the presence of ferrous ions and cysteine molecules in deaerated solutions only the pathways 1 and 2-3 are possible. In this case the cysteine molecules compete for A^\bullet with the ferrous ions. The higher the cysteine concentration the more effective will be the protection of the ferrous ions, which is in good accordance with the experimental results in de-aerated solutions.

In aerated solutions all the pathways are possible. For $c_{\text{Fe}^{2+}} \gg c_{\text{Cy}}$ the pathway 1 is much more probable than pathway 2. But the RS^\bullet -radicals formed by pathway 2 are origins of chain reactions 4-5, by which O_2 is consumed and ferric ions are produced. The higher the cysteine concentration the more probable is pathway 2 and therefore chain reactions 4-5. The quotient Q increases with increasing cysteine concentration. But the quotient Q cannot go to infinity, since there are competing reactions in form of dimerization of RS^\bullet -radicals (pathway 3) and chain reactions 4-6. Consequently, the quotient Q will reach a maximum and then decrease. For $c_{\text{Fe}^{2+}} \ll c_{\text{Cy}}$ only RS^\bullet -radicals are produced (pathway 2), which dimerize or initiate chain reactions 4-6. The result is protection of the ferrous ions.

The investigations of these simple model systems show, that cysteine not only protects against indirect radiation effects, but may cause also sensitization. Sensitization is observed only in the presence of oxygen and depends on the relative concentrations of ferrous ions, cysteine molecules and molecular oxygen as well on the absorbed dose.

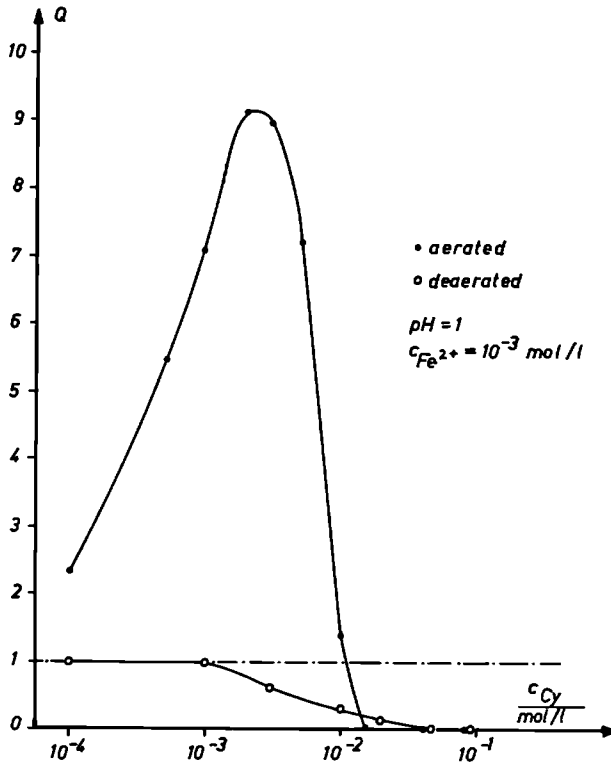


figure 1 : The sensitizing and protective effect of cysteine in acid solutions of ferrous sulphate

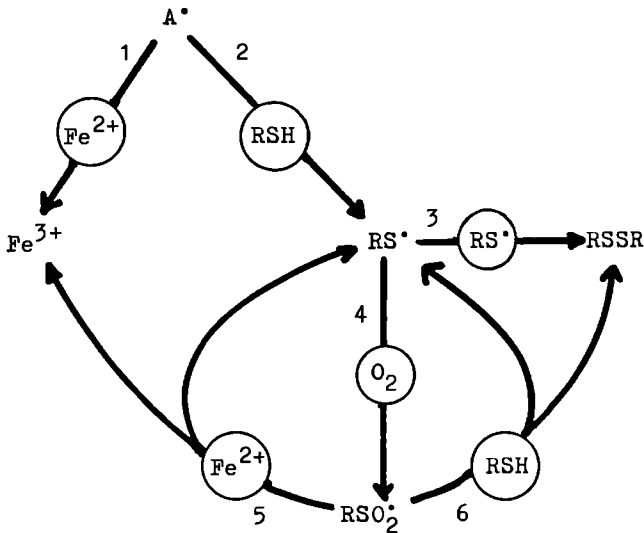


figure 2 : The reaction scheme of irradiated acid solutions with ferrous sulphate and cysteine

Ergebnisse des Projekts Nr. III

Leiter des Projekts und wissenschaftliche

Mitarbeiter: Prof. Dr. W. Pohlit, Dipl.Phys. T. Cremer

Titel des Projekts: TLD in microbiological research

Due to the complexity of the methods used in radiobiology a close cooperation between different laboratories is necessary. This is possible only if the applied absorbed dose in the experiment and other parameters of irradiation such as radiation quality and absorbed dose distribution are well known and comparable. Many of the institutions in this field are not able or are not equipped to performe such measurements with a suitable accuracy. Therefore a dosimetric system is necessary which can be sent by mail from a central station to the interested institution for irradiation. From this system all the necessary parameters must be estimated in a later evaluation of the irradiated probes. The system of choice for this purpose is an arrangement of several thermoluminescent detectors as shown in Fig.3. Up to three different types of radiation fields can be measured with the discs A, B and C. Disc D is for controls only. Each disc is irradiated separately with all the 12 probes in position. In the central position 4 probes are piled up for the determination of the depth absorbed dose as a measure of radiation quality. From these four values also a mean absorbed dose in layers of certain thickness can be determined or the absorbed dose in the surface by extrapolation to thickness zero. The other 8 probes serve for the determination of dose distribution perpendicular to the beam direction.

First LiF-probes have been used for this purpose. But a thermal instability of the trap distribution in this material has been discovered through careful measurements which is called "trap dynamics". Therefore the calibration factor of such probes is not constant enough and LiF is not useful for precise measurements as necessary under these conditions. Other thermoluminescent probe materials have been examined systematically. Up to now only CaF₂ has proved to have traps which are deep enough for dosimetric purposes as shown in table 1 and does not show any trap dynamics. Therefore this probe material will be used for further experiments in the determination of radiation quality for different types of radiations.

Tab.1. Trap depth energy in LiF and CaF₂

Peak No.	Trap depth energy in eV	
	LiF	CaF ₂
1	1.41±0.1	2.0 ±0.1
2	1.77±0.2	2.6 ±0.2
3	2.26±0.2	(3.2)
4	(2.9)	3.7 ±0.4
5	3.62±0.4	4.8 ±0.5

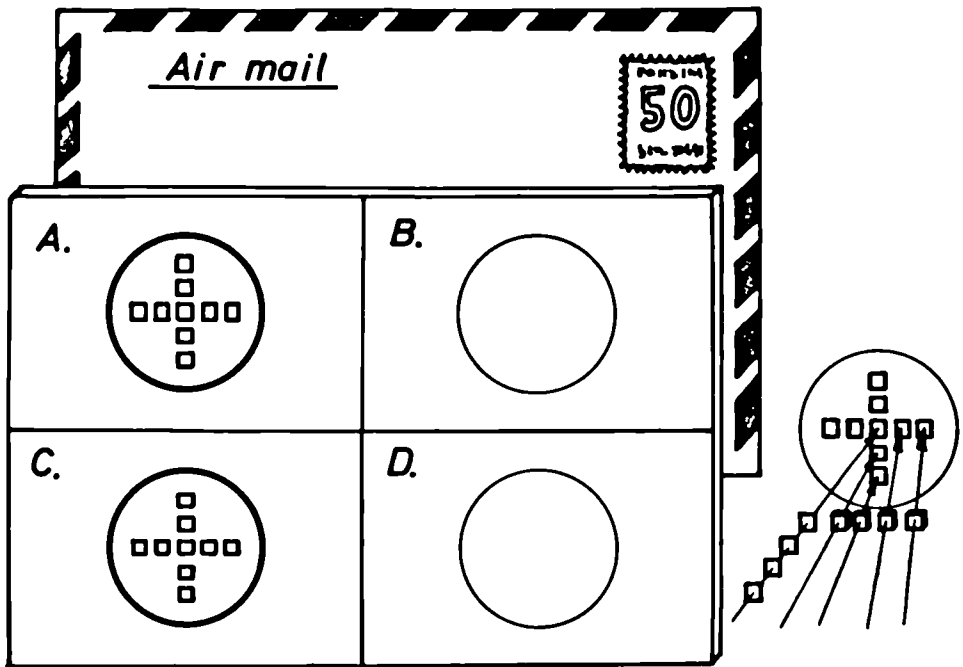


Fig.3 Arrangement of thermoluminescent probes for inter-comparison of dosimetric data by mail.

VERÖFFENTLICHUNGEN

KAPPOS A.

The radial energy distribution of 600 keV protons;
in preparation.

FRANKENBERG D.

The sensitizing and protective effect in X-irradiated model systems containing cysteine; paper presented at the 4th Internat. Congress of Biophysics, Moscow 1972.

POHLIT W.

TLD-probes for intercomparison of dosimetric data; Symposium on dosimetry techniques applied to agriculture, industry, biology and medicine, IAEA/SM-160/7, Vienna, 17-21 April 1972.

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

Nr. des Vertrages: 101-72-1 BIOC

Leiter der Forschungsgruppe: Dr. Georg Burger

Allgemeines Thema des Vertrages: Energieübertragung in Modellsub-
stanzen und Strahleneffekte in kondensierter Materie

The research program includes two projects, both dealing with investigations in the field of radiation quality analysis in the case of neutron irradiation of biological material. Within the first project the event size distributions inside small volumes have been calculated and measured. The calculations are based on a formalism developed by Caswell, the measurements are performed with a Rossi-type counter. In spite of the known short-comings in the theory, regarding the physical input data (cross sections, W-values etc.), the agreement between calculations and measurements is good. Nevertheless, additional MC-calculations have been prepared, for further refined theoretical studies.

The investigations on the ion track analysis have been continued by further improvements of the computer simulations for the delta-ray energy dissipation around the ion path. The second project dealt with the investigation of thermally stimulated exoelectron emission from organic material. The attempts have been continued to analyse several imaginable reasons for the negative results reported earlier. As no exo-emission could be detected the project was stopped and the developed apparatus used for further analysis of TSEE in conventional samples.

Ergebnisse des Projektes Nr. 1.

Leiter des Projektes und wissenschaftliche Mitarbeiter:
G. Burger, G. Leuthold, E. Maier, H. Paretzke

Titel des Projektes: The radiation interaction within tiny
volumes of arbitrary size

Neutron deep penetration calculations have been performed for irradiation of human phantoms (see contract No. 113-72-1 BIOC). Inside the phantom the neutron spectra, the resulting primary charged particle distributions, their slowing down spectra, and finally the event distributions have been calculated following the method of Caswell for a tissue sphere of 2 μm diameter. The measurements have been performed in a low scattering arrangement in the Neuherberg calibration hall.

The neutrons have been produced by bombarding specially prepared thick Tritiumtargets with 400 keV deuterons from a Van De Graaff generator. The neutron peak energy is calculated to be 14.9 MeV, the fwhm around 6%. The elliptical phantom was 22.30·60 cm^3 in size. It was filled with tissue equivalent liquid as proposed by Rossi and Failla. Measurements were performed with and without a neutron collimator. The SSD was 60 cm.

The detector used is a commercially available Rossi-type counter with an inner diameter of 12.7 mm. The pressure of 66.6 Torr simulates a tissue volume of 2 μm diameter of unit density. The counter was either irradiated in free space at the SSD, or positioned along the phantom axis at different depths. In order to demonstrate the accuracy of the calculations, the event desitribution for the Rossi counter used was also calculated for free space irradiation and compared with the measured distribution.

Fig. 1 shows the result with the main reaction components also plotted. The agreement is rather good, considering the limited energy resolution in the measurements.

Folding the theoretical results with a "resolution function" evidently would lower the proton peak and also smear out the α -edge.

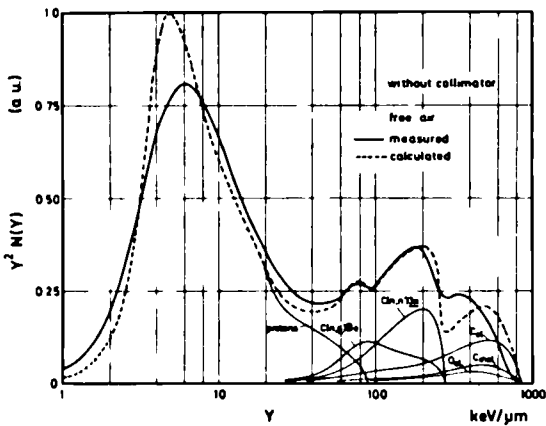


Fig. 1: Measured and calculated $Y \cdot D(Y)$ spectra of 14.9 MeV resp. 15 MeV neutrons.

The main results are the following:

For irradiation of a phantom with 15. MeV neutron without a collimator, the neutron spectra are markedly softened with phantom depth and, as a result, the event distributions change. The main variations occur near the proton edge below an event size of $100 \text{ keV}/\mu$. Using a collimated beam, spectrum hardening and softening along the axis of the phantom cancels each other, so that the event spectra change only slightly.

The event distribution in a tissue sphere is different from that in the equivalent size Rossi counter above about $40 \text{ keV}/\mu$. This is due to the different amounts of oxygen and carbon in both cases.

Calculations of event size distributions in a Rossi-type counter on the basis of Caswell's formalism show sufficient agreement with experimental results for 15 MeV neutron.

Literature:

H. Paretzke, F. Grünauer, E. Maier, G. Burger
 The change of radiation quality in a neutron irradiated phantom
 Proc. First Symp. on Neutron Dos. Biol. Med. EUR 4896 d-f-e, 1972

Ergebnisse des Projektes Nr. 2.

Leiter des Projektes und wissenschaftliche Mitarbeiter:
D.D. Peterson

Titel des Projektes: Study of exoelectrons from solid material

The investigations on plastics have been continued for irradiation with α -particles under vacuum conditions and at liquid nitrogen temperature. Thermal stimulation was then applied. No exoemission could definitely be identified.

For further investigations on the operation of the energy analyzer, it was decided to chose TSEE-materials. The first one was BaSO_4 , the energy distribution of which was previously measured by Kriegseis and Scharmann. The sample is made, as reported there, by suspending BaSO_4 powder in methyl alcohol and placing the mixture onto a thin copper plate. After the alcohol evaporates the powder adheres to the plate which can then be mounted onto the sample platform.

Samples irradiations are done in air with an X-ray machine and an Am^{241} alpha particle source. Following the irradiation, the sample is transferred to the measuring chamber and evacuated. Measurements are taken at a pressure of 4×10^{-6} Torr, with a background signal of 0.1 counts/s.

The results are shown in fig. 1. Curve a) shows the electron transmission curve (i.e. the integral energy spectrum) as a function of the retarding voltage. The maximum exo-electron energy is clearly at most 1 eV.

Curve b) shows the measured derivate curve (i.e. the differential energy spectrum). It is characterized by a maximum near 0,3 eV and a halfwidth of 0,5 eV. These results are the same for alpha and X-ray irradiations in air. They do not agree with those from Kriegseis, who found considerable higher energies.

Further studies have been made in different BeO samples, with and without Li-doping. An example is shown in fig. 2.

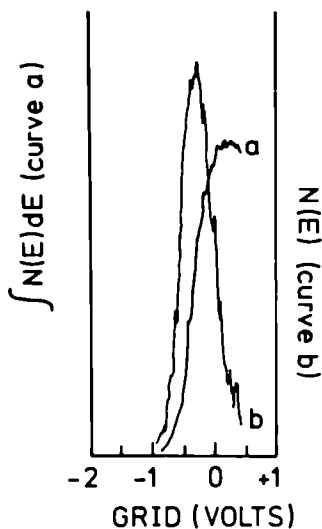


Fig. 1

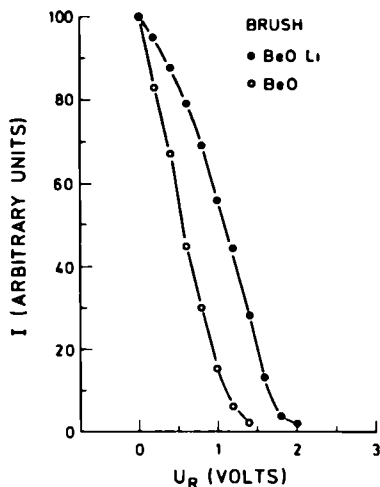


Fig. 2

In this case the doping increases the energy of the emitted electrons. Other commercial BeO-samples showed unexpected high electron energies without doping, which then were decreased by the dopant resulting in the same retarding potential curves than in fig. 2. Summing up it can be seen that Li-doping of BeO-samples "stabilizes" the energy distribution of electrons emitted in the main glow peak at around 400°C.

As a result of our investigations throughout two years and with respect to similar results previously reported by G. Arabin et.al who worked on optical stimulation of exo-emission from plastics, it must be stated, that it seems to be very unlikely to get exoemission from organic material. Due to this, the investigations in plastics will not be continued. The apparatus will be used for further studies in semiconductors, ceramics etc. in the future.

Literature:

- /1/ D.D. Peterson: Energy distribution of thermally stimulated Exo-Electrons from BaSO₄
 phys. stat. sol. (a) 14,K 149 (1972)

Partie A :

- Contractant de la Commission : Université Louis Pasteur de Strasbourg - Laboratoire de Biophysique des Rayonnements et de Méthodologie - 11, rue Humann - Strasbourg
- N° du contrat : 101-72-1-BIOC
- Chef du groupe de recherche : R. RECHENMANN
- Thème général du contrat : Microdosimetry of charged particles in dense matter.

Des protubérances ayant des aspects morphologiques variés ont été observées au microscope électronique le long de traces de particules alpha ($E \leq 8.9$ MeV) enregistrées dans des émulsions ionographiques. Ces couches sensibles avaient été soumises à un des traitements d'activation mis au point en notre laboratoire avant d'être développées d'après une procédure spécifique. Des séries de comptages d'événements ont été réalisées au microscope optique en faisant varier différents paramètres expérimentaux. Par ailleurs, le nombre de protubérances en fonction du parcours croît d'une manière significative en fin et surtout au début de la trace. Tous les résultats de ces études expérimentales excluent la possibilité que les phénomènes observés soient dus à un artéfact photographique.

Parallèlement, une approche théorique a été entreprise en vue de l'interprétation de ces phénomènes. On s'est basé à ce stade de notre étude sur deux hypothèses de travail :

- a) certaines des protubérances correspondraient à des traces courtes d'électrons δ ;
- b) une autre proportion des événements serait causée par des noyaux d'hydrogène éjectés lors du passage de la particule.

Publications : R. Rechenmann, E. Wittendorp. High Efficiency Development Procedures for nuclear emulsions. J1. of Microscopy, Oct. 1972, Vol 96, Pt.2, 227-244.
R. Rechenmann, R. Aiguabella, E. Wittendorp. Energy loss patterns of low-energy α -particles in dense media (en préparation).

Partie B : Résultats du projet n° 1.

Chef du projet et collaborateurs scientifiques : R. RECHENMANN,

R. AIGUABELLA, E. WITTENDORP.

Titre du projet : Study of the energy loss patterns of heavy charged particles.

INTRODUCTION : Nous avons entrepris des recherches systématiques sur les mécanismes de perte d'énergie des ions lourds en fin de parcours, en nous limitant aux phénomènes impliquant une ionisation du milieu. La mise au point de méthodes ionographiques nouvelles résultant en un rendement et une résolution accrus dans l'enregistrement de trajectoires de particules chargées(1), surtout si on les conjugue avec l'observation au microscope électronique, nous a amenés à reprendre l'étude de la structure fine de ces traces. Nous avons ainsi pu observer le long du parcours de particules α des événements que l'observation au microscope électronique permet de résoudre morphologiquement en des élargissements très localisés de la trace ou en des embranchements de longueurs et d'épaisseurs variables, formant avec l'axe de la trace des angles variés (2) (Fig.1 et 2).

I. METHODOLOGIE : Les émulsions ionographiques (Ilford K5, K2, L4, Montreal B55) ont été exposées sous une incidence rasante à une source de ThB (particules α respectivement de 6.2 MeV du ThC, et de 8.9 MeV du ThC'). Après activation à l'or de l'image latente (1), elles ont été développées soit dans l'amidol, soit dans différents types de révélateurs à base d'oxalate ferreux.

1°) Dans cette première phase de notre étude, le microscope électronique a été utilisé surtout pour l'observation de la morphologie des événements. Nous avons également mesuré la longueur moyenne des embranchements, qui est de l'ordre de $0.5 \mu\text{m}$ dans l'émulsion Ilford L4. 2°) On a déterminé au microscope optique la fréquence des protubérances en fonction de différents paramètres expérimentaux, notamment de la taille moyenne des microcristaux. Le pourcentage des protubérances augmente si le diamètre du grain diminue; ceci favorise l'hypothèse selon laquelle les embranchements seraient des traces de particules chargées ayant des parcours définis dans le système gélatine-BrAg. Nous avons par ailleurs déterminé les histogrammes du nombre de protubérances en discriminant entre les embranchements ayant une longueur projective (perpendiculairement à l'axe de la trace) plus petite ou égale à $0.5 \mu\text{m}$, et ceux ayant une portée supérieure à $0.5 \mu\text{m}$ (Fig.3 et 4).

II. CONSIDERATIONS THEORIQUES. Nous nous sommes basés sur deux premières hypothèses de travail pour interpréter les événements considérés : 1°) Les protubérances seraient partiellement dues à des électrons éjectés lors de collisions ionisantes avec les atomes du milieu. Le calcul de la probabilité d'éjection d'un électron en lère approximation de Born implique a) des perturbations électromagnétiques très faibles des orbitaux, et b) des vitesses β de la particule chargée (ze) incidente telles que $\beta \gg Z_1 Z_2$ (3). Pour des atomes lourds (Br-Ag) dont la charge effective nucléaire agis-

sant sur un électron Z' est $Z' \ll Z$ la condition a) est satisfaite et s'avère suffisante pour assurer la validité de la 1ère approximation de Born (4,5). Nous avons donc utilisé le traitement de Merzbacher et coll. (6) pour obtenir le taux linéaire d'électrons éjectés lors des collisions de particules avec les orbitaux fortement liés des atomes Br et Ag. En ce qui concerne la composante légère de l'émulsion (C-N-O-H) où $0.7 \ll Z' \ll 7.7$, on doit s'attendre à des effets de polarisation des orbitaux (6). Les conditions a) et b) n'étant pas remplies, l'applicabilité de la 1ère approximation de Born n'est pas rigoureusement assurée. Dans cette première approche théorique, nous avons néanmoins calculé d'après Bhabha (7), donc en 1ère approximation de Born, le nombre de particules ayant une énergie $T \gg T_0$, à condition que T_0 soit beaucoup plus grand que l'énergie moyenne de liaison des électrons.

Au stade méthodologique actuel, seuls les électrons ayant une énergie $T \approx 4-5$ KeV pouvant être décelés, nous avons tenu compte de ce seuil dans les calculs des taux linéaires respectifs d'électrons d'ionisation pour les atomes Ag-Br et C-N-O-H (Fig.5).

2° La deuxième hypothèse de travail concerne la possibilité d'interactions élastiques entre la particule incidente de masse M et les noyaux atomiques M_1 pour un rapport M/M_1 suffisamment grand. A partir de la formule classique de Rutherford, corrigée pour tenir compte des effets d'écran ainsi que de la faible énergie de la particule (8), nous avons calculé le taux linéaire de noyaux d'atomes d'hydrogène émis, dans l'émulsion standard, en fonction de l'énergie de la particule (Fig.6).

III CONCLUSION . Les premiers résultats excluent absolument les possibilités d'artéfacts photographiques ou autres à l'origine des protubérances le long des traces. Par contre, nos hypothèses de travail, à savoir que le plus grand nombre des embranchements seraient dus à la fois à des rayons δ et à des protons (surtout en début de parcours résiduel), semblent donner une description cohérente de nos premiers résultats expérimentaux. Finalement, il faut envisager la possibilité selon laquelle d'autres mécanismes d'interaction des particules avec la matière dense seraient à l'origine des phénomènes considérés.

- 1) RECHENMANN R., Proc.7th. Int.Coll.Corpusc.Phot.and Visual Solid Detectors, Barcelona 1970; Rapport Euratom N°EUR.4688e, 1971.
- 2) RECHENMANN R., WITTENDORP E., 39ème Congrès Ann. ACFAS, Sherbrooke, oct.1971.
- 3) WILLIAMS E.J., Rev.Mod.Phys. 17, 217, 1945.
- 4) MOTT N.F., Proc.Cambridge Phil.Soc. 27, 553, 1931.
- 5) HENNEBERG W.Z. Physik 86, 592, 1933.
- 6) MERZBACHER E., LEWIS H.W., Handbuch der Physik XXXIV, 166, 1958.
- 7) BHABHA H.J., Proc.Roy.Soc.London, A164, 257, 1938.
- 8) LITTON G.M., LYMAN V. et TOBIAS C.A., UCRL-17392, Berkeley, Cal., 1968.

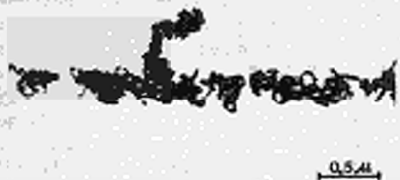


Fig. 1



Fig. 2

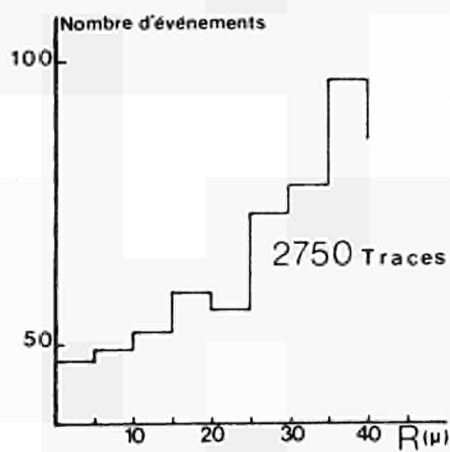


Fig. 3

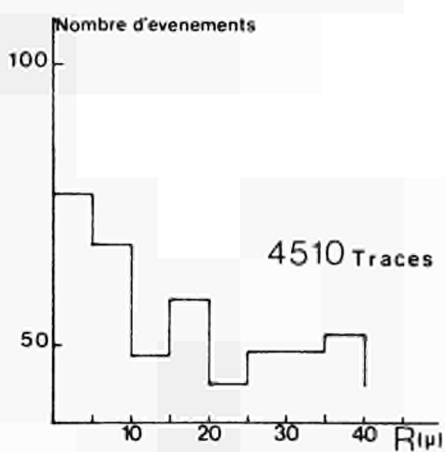


Fig. 4

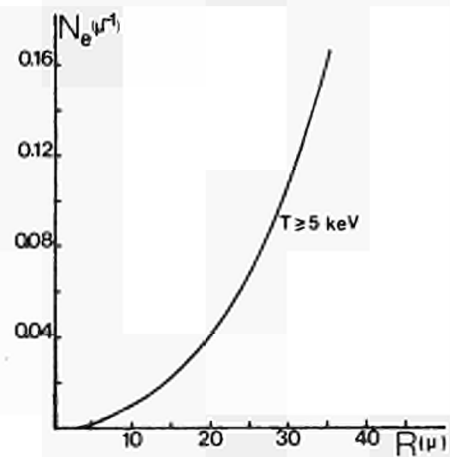


Fig. 5

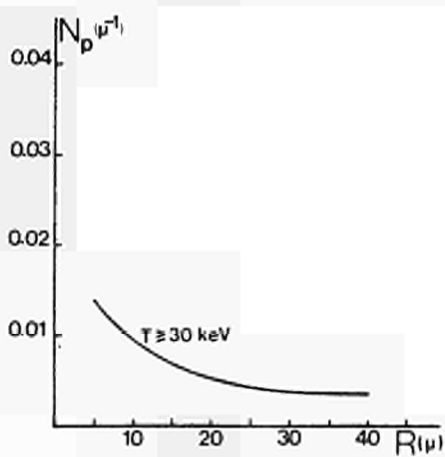


Fig. 6

Vertragspartner der Kommission: Institut für Biophysik,
Homburg/Saar, Universität des Saarlandes

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

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

Allgemeines Thema des Vertrages: Energy Transfer in Biological Material and in Model Substances.

It is the purpose of this work, to get a little more insight into the processes of energy transfer which lead after absorption of ionizing radiation energy to biological radiation damage at or near by the molecular level. Therefore we investigated the chromosome aberration rates as indicators of radiation damage near by the molecular level and the behaviour of radiation induced radicals. For measuring the radicals it is necessary to freeze in the samples. By this procedure the life time of the radicals is lengthened and the absorption of micro wave power (ESR-spectroscopy) is avoided. Further more the subsequent slowly increase of temperature enable us to watch the radical reactions in the sample.

We now have established the chromosome aberration rates for cells irradiated at 77°K . After measuring the radicals produced in water as well as in complete cell suspensions we try to get the contribution of the most important cell compounds to the whole ESR-spectrum.

From earlier measurements we could learn, that some of the primary radiolytic water products are not longer present at 77°K . Therefore we introduced radical scavengers into our experiments and prepared investigations at much lower temperatures then 77°K (liquid helium) to make the radiolytic products more stabile.

Furthermore we have started the investigation of ESR-spectra produced by radiation of higher LET (α -rays of ^{210}Po) to see if there are differences in spectra compared to those caused by X-ray produced radicals.

Results of Project Nr. 1

Leader of the project and scientific coworker:

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

Title of the project:

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

1. The earlier investigations of lymphocytes which have been cooled down at 77°K and which were irradiated at the same temperature showed that the chromosome aberration rates of the various types are lowered by a factor of 15 (resp. 45) compared with cells irradiated at room temperature. Because such a large "radiation protection factor" has not been observed up to now, these investigations were repeated during the past year at doses starting at 500 R to 5000 R in steps of 500 R. The new mean values are in agreement with the former ones within the variation limits caused by the statistical uncertainties and the biological variability. The publication of these results is in preparation and will appear early in 1973.

2. In connection with the investigations of the chromosome aberrations rates the frequencies of cells with 0, 1, 2 and more aberrated chromosomes in dependence on radiation dose were determined. The points giving the numbers of cells with 0, 1, 2 and so on aberrated chromosomes for a fixed dose have still to be fitted with the appropriate distribution curve. It can be seen, that if one puts on the ordinate the numbers of aberrated chromosomes in a cell which appear most frequently and on the abscissa the radiation dose, the points on a first approximation are fitted by a straight line. The discussion of these results is still going on but they also will be published early in 1973.

3. In preparing the investigations of radicals and chromosome aberrations, which appear after irradiation at the temperature of liquid helium, lymphocyte cultures were irradiated.

ted at $+4^{\circ}\text{C}$ with β -rays of tritium instead of X-rays. For this purposes tritiated water was added to the cellsuspension. This procedure has the advantage that technically complicated apparatuses for keeping the low temperature of 4°K during irradiation and transport of the samples from the locus of irradiation to the sample container of the ESR spectrometer can be avoided. The preliminary investigations showed, that at the same doses but at much smaller dose-rate (irradiation time: X-rays about 2 min, β -rays 20 hs) the chromosome aberration rate caused by the β -rays was only 50 % of that caused by X-rays (X-ray dose was measured in R, β -ray dose was calculated from mean β -ray energy, ^3H -activity and exposure time in Rad) For further investigations the specific activity of tritium was taken 10 times as high as before, to get a higher dose rate. The exploitation of this experiments has not been finished till now.

4. In continuing the investigations of radicals which are present after irradiation at 77°K , solutions of pure DNS and Na-DNS salts in water were frozen and irradiated and the behaviour of the radicals in the range of 90°K to the melting point was investigated. Similar spectra and similar behaviour like those after irradiation of complete cell suspensions were found. Above all in these solutions the share of the organic radicals of the whole spectra is also larger then the share of the organic material in the solution. The final exploitation of these results has still to be carried out.

5. To catch the water radicals which at 77°K are instable, experiments with solutions of tryptophane as a radical scavenger were started. The preliminary results showed the expected behaviour: At first a higher share of tryptophane radicals as should be found according to the share of tryptophane of the whole sample mass (propably caused by reaction of H^{\cdot} - and e^{-} -radicals with tryptophane radicals) and than an increase of triptophane radicals with increa-

sing temperature, when the OH' radicals get back their mobility.

6. To see the influence of LET in further experiments the radicals produced by ^{210}Po α -rays in water were investigated. Unfortunately no carrier free α -ray emitter soluble in distilled water (at pH = 7) was available. Therefore the exploration of the spectra is difficult. Also a direct treating of cells with this radionuclid (Po-Nitrate in 3 M - HNO_3 solution) is not possible.

Before going on in these experiments an appropriate commercially available α -ray emitter must be found.

Associato della Commissione: Laboratorio di Dosimetria e Standardizzazione

N° del contratto: 068-67 BIOD

Capo del gruppo di ricerca: Prof. E. Casnati

Tema generale del contratto: Application of solid state devices to radiation dosimetry

In the original research program 1972-1974 the three following projects were included:

- a) application of solid state devices to dose intercomparisons;
- b) dosimetry of mixed fields of neutrons and gammas;
- c) use of TSEE solid detector in the dosimetry at interfaces between different media.

In 1972 only the first project has been developed with the results described in the next page.

This was due to the fact that the staff (1 graduate and 1 technician) and the financial support necessary to carry out the above mentioned program, has not been officially approved during this year.

Risultati del progetto 1

Capo del progetto: Prof. G. Scarpa

Titolo del progetto: Application of solid state devices to dose intercomparison

A large scale practical application of TLD's in intercomparison has been carried out at the last dose intercomparison promoted by EULEP. Lithium fluoride as loose powder has been used in the first part of this study.

The problem of intercomparisons has been discussed during the meeting organized by EULEP at Rijswijk and the one held in Toulouse by the Dosimetry Group of EURATOM.

Members of this Laboratory have taken part in these meetings where the EULEP Dosimetry Group came to the decision to perform a second intercomparison to check the improvements made on the dosimetry at the various Institutes. This project will begin next February and measurements will be carried out both with LiF powder and with beryllium oxide sintered dosimeters. Everything concerning preparation, package, final read out of BeO disks will be done in this Laboratory.

In order to investigate specifically the use of ceramic BeO as postal TL dosimeter, and additional study has been performed with special regard to the following characteristics: disc-to-disc response, fading, linearity, energy dependence and some specific problems connected with postal delivery.

As previously published, the use of unselected discs belonging to the same batch gives a standard deviation of TL responses ranging from 6 to 7 percent. In order to minimize this kind of error two methods can be used: first, the pencil marking of each single disc followed by an individual calibration; second, the preliminary selection of the discs whose sensitivity ranges between stated limits,

e.g. 1 or 2 percent. This second technique was selected as the most convenient one. A set of experiments have shown that a stable standard deviation of $\pm 3\%$ can be easily attained; this figure looks quite acceptable as, basing on pure statistical laws, the mean value of 4 readouts would fluctuate by only $\pm 1.5\%$.

The fading was stated in the same conditions met during a real intercomparison, i.e. with a variable annealing-to-irradiation and irradiation-to-readout time lag. The relative response of the dosimeters has shown a variability not exceeding $\pm 2\%$. This low figure is probably due to a sort of compensation between fading and rise of sensitivity after annealing.

The supralinearity of BeO was found to be less marked for X-rays than for gamma rays. The linearity index reaches its maximum value at about 1000 rads for both radiation qualities.

The energy dependence was studied with special regard to X-rays in the range of 1 to 3 mm Cu HVL, i.e. the energy range used in most radiobiological experiments. The variation of the relative response is between 7 and 17%, according to dose level: this variability is due to the dependence of supralinearity index on the radiation energy.

In order to ensure a correct delivery of the material throughout the intercomparison, a special plastic container has been planned, made of antishock black polystyrene. This container is completely light-tight, shock-proof and open-proof.

Bibliografia

- (1) G. Scarpa, G. Benincasa, L. Ceravolo - The Thermoluminescence of Beryllium Oxide as applied to Radiation Dosimetry. Submittet to Symposium on Dosimetry Techniques Applied to Agriculture, Industry, Biology and Medicine. Vienna, 17-21 April 1972.

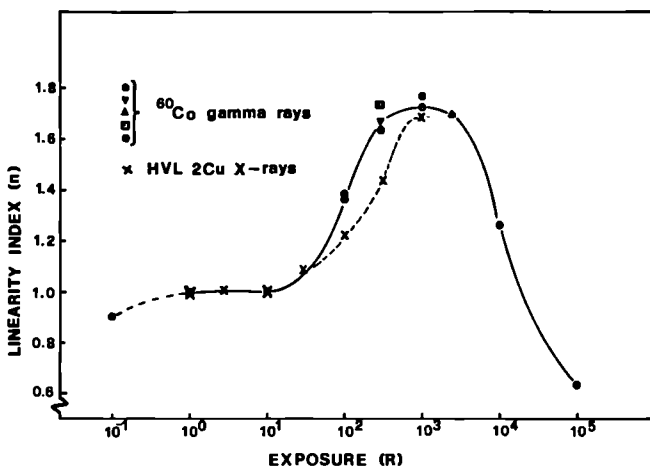


Figure 1

Behaviour of linearity index vs. exposure level and quality of radiation for BeO TL dosimeters.

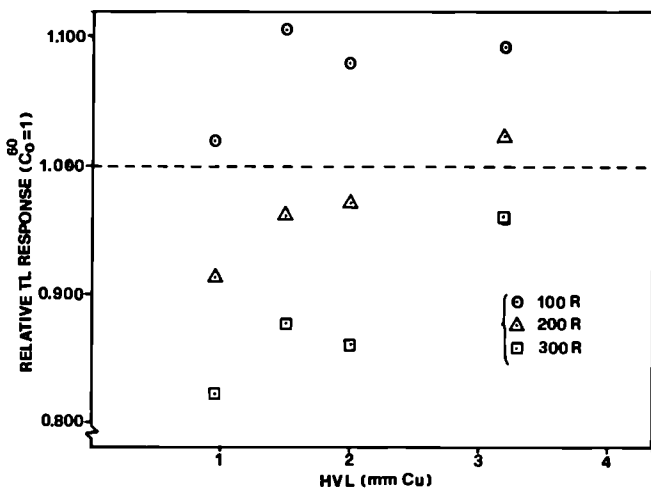


Figure 2

Energy dependence of BeO TL dosimeters at various exposure levels in the range between 1-3 mm Cu HVL.

Annual Report 1972
of the group contract on personnel dosimetry

For the Health Protection Directorate the personnel dosimetry group contract (contract no. 065-72-1 PST C) constitute a widening and completion of efforts to establish basic standards for the protection of the population and labour force against the health hazards of ionising radiation. This agreement is furthermore an important contribution to the promotion of research and standardisation in the field of radiation protection within the European Community.

Research groups from the following institutions are members of the agreement :

- Comitato Nazionale per l'Energia Nucleare, Roma ;
- Commissariat à l'Energie Atomique, Paris ;
- Gesellschaft für Strahlen- und Umweltforschung,
Neuherberg/München ;
- Kernforschungsanlage Jülich GmbH, Jülich.

The aim of the agreement is to improve existing methods and to develop new ones. The following principal domains are treated in the group agreement : neutron dosimetry, standardisation and calibration, nuclear track etching, dosimetry in mixed radiation fields, x-ray luminescent radiation for calibration purposes and stimulated exoelectron emission.

In the interests of close cooperation, partners in the appropriate areas of the field were annually called to a meeting to report on results achieved, exchange notes and discuss further research projects. Bilateral discussions were held between the partners to agreement. In order to enable the partners under the research agreement to take account of practical requirements, they were invited to attend meetings of the technical experts at which the implementation and

findings of the intercomparison programme are discussed. Knowledge and experience gained from practical observation thus directly reflected in the execution of research work.

In order to make the results of work under this agreement available to a wider circle, establish contacts and receive suggestions, eight papers based on work promoted by the Health Protection Directorate were presented at two international symposia (first symposium on neutron dosimetry in biology and medicine, Neuherberg/München ; symposium on neutron monitoring for radiation protection purposes, Wien).

Contrat N° 65 - 72 - 1 PSTC

Organisme - lieu - pays - Commissariat à l'Energie Atomique - Centre d'Etudes
Nucléaires de FONTENAY-aux-ROSES - FRANCE -

Chef du Groupe de recherche - G. SOUDAIN

Chef du projet - G. PORTAL - J.L. CHARTIER

Thème général - Production de rayonnements photoniques de référence pour la dosi-
métrie.

Description générale succincte des travaux accomplis :

L'étude de la production des rayonnements monochromatiques d'énergie inférieure à 250 keV à l'aide d'un spectromètre à cristal courbé a été poursuivie en 1972.

I - EMPILEMENTS DE CRISTAUX -

Rappelons que nous avons constaté en 1971 qu'il n'était pas possible de courber des cristaux de fluorure de lithium d'épaisseur supérieure à 4 mm sans altérer exagérément la qualité des rayonnements obtenus. Or, il est nécessaire d'utiliser des cristaux plus épais si l'on veut disposer de faisceaux de rayonnement X suffisamment intenses.

Nous avons essayé d'empiler plusieurs cristaux de fluorure de lithium convenablement orientés. Nous avons construit un dispositif pour le calage de ces cristaux et mis au point un système d'orientation très précis.

Les résultats obtenus sont tout à fait satisfaisants. En effet, on obtient à titre d'exemple :

- une résolution de 2 keV pour un rayonnement de 50 keV
- une résolution de 5 keV pour un rayonnement de 100 keV
- des débits variant de 250 à 400 mrad/heure pour un tube à rayons X fonctionnant sous une intensité de 10 milliampères.

II - NATURE DU CRISTAL -

Nous avons également essayé d'accroître l'intensité du faisceau en utilisant des cristaux dont le facteur de structure et le coefficient de réflexion sont supérieurs à ceux du fluorure de lithium.

Les cristaux d'aluminium qui ont été fabriqués à notre demande ne nous ont pas donné satisfaction; en effet l'état de surface n'est pas de bonne qualité et l'on n'a pas encore trouvé de méthode de polissage qui soit efficace et qui n'altère pas les propriétés des cristaux.

Le germanium nous a donné de bons résultats ; bien que l'on n'ait pas essayé de courber plastiquement des lames d'épaisseur supérieure à 1 mm, les débits obtenus sont identiques à ceux cités dans le cas du fluorure de lithium. Cependant le germanium présente sur ce dernier un avantage considérable : la diffusion d'ordre 2 présentant une intensité comparable à celle d'ordre 1, il est parfaitement possible d'obtenir des rayonnements de 200 keV, alors qu'avec le fluorure de lithium on est pratiquement limité à 100 keV. Les essais d'empilement seront entrepris prochainement.

Le cuivre devrait permettre d'obtenir des résultats encore supérieurs. En effet son facteur de structure est 4 à 5 fois plus élevé que celui du germanium. Nous nous heurtons pour l'instant, tout comme pour l'aluminium, au problème de l'état de surface. Des études sont en cours pour le résoudre. Nous comptons notamment effectuer un polissage chimique.

III - TUBE A FOYER VARIABLE -

Nous avons fait réaliser un tube à foyer variable permettant d'adapter la dimension de la source d'émission de rayonnement à celle de la fente définissant la résolution du faisceau afin d'augmenter la "brillance" relative de la source de rayonnement. Les essais ont montré que nos prévisions étaient justifiées : à résolution égale on obtient un débit d'exposition dans le faisceau deux fois plus important quand on supprime la fente.

L'étude de l'optimisation des caractéristiques de cette installation se poursuivra en 1973 et nous fondons les plus grands espoirs sur l'utilisation de cristaux de germanium et de cuivre.

PUBLICATION - J.L. CHARTIER, G. PORTAL, D. ROMAN, D. DUGAY.

Production de rayonnements monochromatiques intenses - Nuclear Instr. and Meth. 100 (1972) 107.

CONTRATTO : 065-72-1

G. BUSUOLI

STUDIO DI NUOVI RIVELATORI APPLICABILI ALLA DOSIMETRIA PERSONALE

L'attività portata avanti in questo laboratorio in connessione al contratto in oggetto ha riguardato i seguenti due argomenti:

- 1) Applicabilità dei rivelatori TSEE alla dosimetria personale.
In questo ambito sono state condotte ulteriori prove di riproducibilità sul BeO che in linea di massima si sono dimostrate più positive rispetto a quelle analoghe portate a termine nel corso dell'anno precedente. Si è notato inoltre che la riproducibilità dipende dalla geometria del rivelatore utilizzato.
- 2) Applicabilità dei rivelatori plastici a tracce nella dosimetria da incidente di criticità. Nelle prime prove condotte si è studiata in modo sistematico l'influenza del tempo e della temperatura d'attacco delle plastiche sul numero di tracce rivelabili. Inoltre si è rivolta particolare attenzione allo angolo limite di rivelazione al di sotto del quale le particelle cariche non vengono più rivelate e ciò in relazione alla utilizzazione di schermi convertitori di Boro o di Litio per la rivelazione dei neutroni.

PROGETTO N. 1

G. BUSUOLI, L. LEMBO

STUDIO DELLA APPLICABILITA' DEI RIVELATORI TSEE ALLA DOSIMETRIA
PERSONALE

L'attività condotta nel Laboratorio di Fisica Sanitaria di Bologna sul BeO ha riguardato principalmente i seguenti due aspetti:

- a) riproducibilità;
- b) studio della risposta TL del BeO in vista di una utilizzazione combinata dei due effetti nell'impiego del rivelatore stesso.

Il punto a) è stato un proseguimento delle prove condotte nel corso del 1971 dato che queste avevano ancora una volta mostrato che lo scoglio maggiore per il proseguimento della attività derivava proprio dalla scarsa riproducibilità del rivelatore usato come TSEE.

Riproducibilità

Visti i risultati non molto soddisfacenti raggiunti nel corso del 1971, per quanto riguarda la riproducibilità è stata rivolta in un primo tempo particolare attenzione alla messa a punto del contatore che come si ricorderà è un G.M. a flusso di elio isobutano. Per questo si è leggermente modificato il fornello riscaldante ed inoltre si sono eseguite prove di funzionamento del contatore cercando di ottenere le condizioni di miglior impiego. Si pensa inoltre di sperimentare altri tipi di contatori (come ad esempio quello a punta già in corso di allestimento) in relazione all'elevato tempo morto del G.M. ora utilizzato.

Dopo queste prove preliminari si è passati alle prove di riproducibilità con le pastiglie di BeO. Queste sono state

sempre irraggiate, senza togliere dal contatore, con una sorgente di Am^{241} in modo da eliminare eventuali effetti spuri durante il trasporto del rivelatore. Ovviamente queste non possono essere le condizioni per verificare la riproducibilità, ma le prove così condotte hanno avuto lo scopo di verificare se la riproducibilità era dovuta a cause intrinseche alle pastiglie oppure alla manipolazione delle stesse. In queste condizioni sono state condotte prove con pastiglie di BeO (Thermolox 995) di diverso diametro e si è arrivati alla conclusione che la riproducibilità è una funzione del diametro della pastiglia stessa. A conferma di quanto accennato in precedenza, in Tab.1 sono riassunti i risultati delle prove con le pastiglie di diverso diametro.

TABELLA 1

N° pastiglia	Diametro pastiglia			
	22,35 mm	15,85 mm	12,75 mm	8,75 mm
1	$\sigma = 24\%$	$\sigma = 18\%$	$\sigma = 22\%$	$\sigma = 5\%$
2	$\sigma = 55\%$	$\sigma = 27\%$	$\sigma = 7\%$	$\sigma = 4\%$
3	$\sigma = 36\%$	$\sigma = 18\%$	$\sigma = 12\%$	$\sigma = 6\%$

Nota: Le dosi sono state sempre equivalenti ad 1 rad di Co-60 . La σ si riferisce a 7 misure eseguite sulla stessa pastiglia.

Non è facile dare una interpretazione di questo fenomeno; un semplice tentativo può essere quello di attribuire la migliore riproducibilità al fatto che le pastiglie più piccole introducono minori distorsioni delle linee di forza del campo elettrico all'interno del contatore. Bisogna infatti ricordare

che i rivelatori sono dei perfetti isolanti e sono sempre stati utilizzati senza renderli conduttori in superficie mediante la deposizione di uno strato di grafite o di un metallo a numero atomico più elevato.

Contemporaneamente sono state condotte prove di termoluminescenza con il BeO ceramico per poter fare un confronto con le prove sugli esoelettroni.

Utilizzati in questo modo i rivelatori si comportano in modo migliore. Infatti su un totale di 180 misure ripetute su 30 diverse pastiglie e per una esposizione di 200 mR si è avuta una standard deviation del $\pm 7\%$ senza usare particolari precauzioni durante gli irraggiamenti e le letture. La riproducibilità su una stessa pastiglia risulta (sempre a 200 mR) ancora migliore e dell'ordine di qualche per cento.

Anche usando il BeO come termoluminescente si è riscontrato che la riproducibilità dipende dal diametro delle pastiglie utilizzate non però in maniera così semplice come avviene nelle misure esoelettroniche. Questo tipo di fenomeno è piuttosto strano e potrebbe essere dovuto, in questo caso essendo un fenomeno di volume, ad una diversa distribuzione delle trappole derivante da eventuali differenze nel procedimento di preparazione delle pastiglie.

PROGETTO N. 2

A.CAVALLINI, A.FASSO'

APPLICABILITA' DEI RIVELATORI PLASTICI A TRACCE NELLA DOSIMETRIA
DA INCIDENTE DI CRITICITA'

La ricerca ha lo scopo di preparare un dosimetro per neutroni basato su rivelatori plastici a tracce. Ci si è orientati sulla reazione (n, alfa) nel Boro o nel Litio, perchè consente, in linea di massima, di rivelare neutroni di tutte le energie che si possono avere in caso di incidente (dai neutroni termici fino a qualche MeV). Per la discriminazione di un certo numero di bande di energia, s'è pensato di utilizzare schermi di Boro per le energie basse e intermedie, e una spettrometria alfa grossolana con filtri sottili per le energie veloci.

Misure effettuate

Come rivelatore si è scelto il nitrato di cellulosa per la sua sensibilità e perchè viene fornito in commercio con caratteristiche riproducibili. Come attacco chimico s'è usato un bagno pure reperibile in commercio (Kodak VX-007).

Sono state eseguite diverse serie di misure allo scopo di definire esattamente le caratteristiche del materiale prescelto.

Si è determinato, per sorgenti alfa di diversa energia, l'andamento in funzione del tempo di attacco del numero di tracce rivelate. Per le sorgenti la cui energia massima è superiore a E_2 , dove E_2 è l'energia oltre la quale il valore di $\frac{dE}{dx}$ scende sotto la soglia di rivelabilità, si ha un aumento grosso modo lineare delle tracce rivelate in funzione dell'attacco chimico, fino a un valore massimo di saturazione, che rimane poi costante.

Per le sorgenti la cui energia massima è inferiore a E_2 , il numero di tracce rivelato è costante, qualunque sia il tempo di attacco. A una data temperatura, il tempo occorrente per avere saturazione è il tempo necessario per asportare uno spessore di plastica uguale alla differenza di percorso delle alfa di massima energia della sorgente e le alfa di energia E_2 . Confrontando tali tempi ottenuti con due sorgenti di energia massima diversa, si ottiene il valore di E_2 , e quindi anche il tasso critico di perdita di energia $(\frac{dE}{dx})_c$.

Come sorgenti per questa serie di misure abbiamo utilizzato una sorgente di Np^{237} nuda, e la medesima sorgente coperta con un certo numero di film sottili. (Mylar di $6 \mu m$ di spessore).

La stessa serie di misure ha consentito di stimare un limite superiore dell'angolo di incidenza critico, oltre il quale le particelle alfa non vengono registrate. A causa degli errori non è stato però ancora possibile determinare il valore esatto, e a questo scopo sono in corso ulteriori misure. Si è trovato che l'angolo in questione rispetto alla superficie della plastica è inferiore a 30° (MOURGUES (1) dà per l'angolo limite il valore 28°).

E' stata anche determinata l'influenza della temperatura del bagno sui risultati. Il tempo necessario per giungere a saturazione varia colla temperatura del bagno, ma il valore di saturazione rimane invariato. Ciò contrasta con quanto afferma la Ditta produttrice del nitrato di cellulosa, secondo la quale, per temperature superiori a $60^\circ C$, si avrebbe un considerevole "fading" in competizione col processo di rivelazione.

Tutte queste misure preliminari sono necessarie, perchè il metodo che si è scelto per la rivelazione dei neutroni (conversione per reazione (n, α) su Litio o su Boro) implica una completa conoscenza delle caratteristiche del rivelatore

per ciò che concerne la dipendenza dall'angolo di incidenza e dall'energia delle particelle. Parallelamente al lavoro sperimentale, è stata svolta anche un'attività di calcolo (perdita di energia, percorso delle alfa, assorbimento), necessaria per l'interpretazione delle misure.

- (1) M.MOURGUES - Contribution à l'étude de l'enregistrement des traces des particules alpha dans les détecteurs de nitrate de cellulose.
Tesi - Centre d'Etudes Nucléaires de Cadarache, Sept.1970

Contrat N° 065-72-1 PSTC

Commissariat à l'Energie Atomique (FRANCE)

M. BRICKA *

Description générale succincte des travaux accomplis :

L'effort le plus important a été fait en 1972 sur le développement d'un compteur à hélium 3 présentant des caractéristiques convenables pour l'utilisation sous sphères de polyéthylène.

Les prototypes dits à fenêtre céramique réalisés par LMT, actuellement à l'essai, ont une excellente isotropie et un rendement supérieur à celui des cristaux d'iodure de lithium qu'ils sont destinés à remplacer. Une matrice des réponses des sphères équipées de ce compteur a été déterminée par le calcul en utilisant la théorie du transport multigroupe (codes ANISN et DTF IV). Cette matrice sera ultérieurement recalée sur des mesures en neutrons monoénergétiques.

La détermination des courbes de réponse pour les sphères équipées de cristaux d'iodure de lithium a été poursuivie. La matrice des réponses a été obtenue en combinant les données des mesures en neutrons monoénergétiques avec celles du calcul.

Cette matrice a été utilisée pour divers essais de spectrométrie et donne des résultats parfaitement cohérents avec ceux qui ont été obtenus, par ailleurs avec des détecteurs à activation. Les premiers essais de spectrométrie réalisés auprès du convertisseur 14 McV-fission montrent également une bonne concordance entre les spectres donnés par le multisphère et par le compteur proportionnel à protons de recul.

Une communication faisant le point de ces travaux est présentée au colloque sur la dosimétrie des neutrons (VIENNE - DEC. 72). (1)

Ces études sur les sphères modératrices sont menées en liaison avec le Dr HEINZELMANN. Une réunion de coordination a été tenue à CADARACHE entre les chercheurs de KFA et ceux du CEA (Mai 72).

* Département de Sécurité Nucléaire - Service d'Etudes de Sécurité Radiologique - CEN/CADARACHE.

(1) BRICKA et Coll.
VIENNE 1972 - Rapport IAEA - SM 167/19.



Titre : Développement d'un compteur à hélium 3.

Noms des chercheurs : J. CERCY, R. LORIENT

Description des résultats :

Le compteur à hélium est destiné à remplacer les cristaux d'iodure de lithium trop sensibles au rayonnement gamma.

Un premier prototype LMT 0,4 NH 1/1 (figure 1) avait été réalisé sous l'impulsion de H. VIALETES. Ce prototype du fait de la position du queusot présentait une assez forte anisotropie et son montage dans les sphères de petit diamètre posait quelques problèmes.

Dans les nouveaux prototypes LMT 0,5 NH 1/1, dits à fenêtre céramique, le volume sensible se trouve à l'extrémité du compteur. L'isotropie est ainsi améliorée (< 10%) et le montage sous sphère facilité.

Le remplissage de ces compteurs est constitué par un mélange

hélium 3 : 8 bars krypton : 2 bars Autocoupeur : ξ

La figure 2 présente les courbes de réponse en fonction de la tension obtenues pour deux des prototypes essayés, avec un gain de 58 db et un seuil de 0,5 volts.

Le premier compteur à fenêtre céramique réalisé avait une dérive importante - de l'ordre de 30% - à la mise sous tension. Une modification de la fenêtre céramique a permis de réduire à moins de 10% cette dérive - quinze minutes après application de la haute tension le compteur reste parfaitement stable.

Dans le prototype le plus récent, actuellement à l'essai, et dont la géométrie est un peu différente de celle des précédents, on ne constate plus aucun phénomène de dérive.

Le rendement des divers modèles du type 0,5 NH 1/1 est légèrement inférieur à celui du premier prototype 0,4 NH 1/1. Il reste cependant supérieur au rendement du cristal d'iodure de lithium.

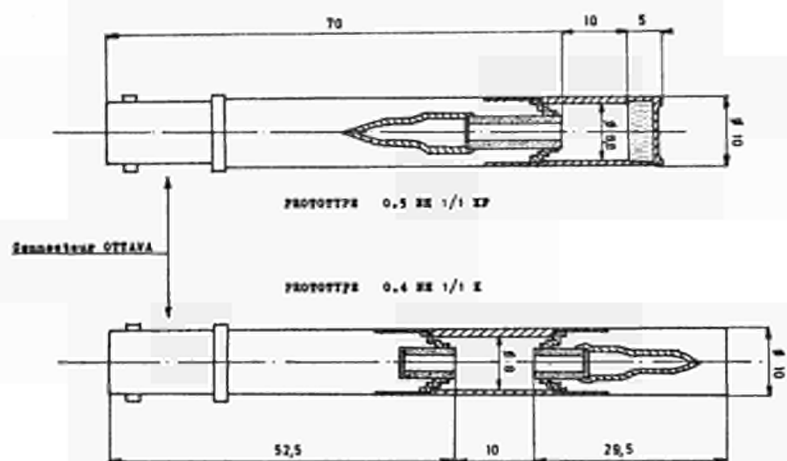


FIGURE 1

ECHELLE 2

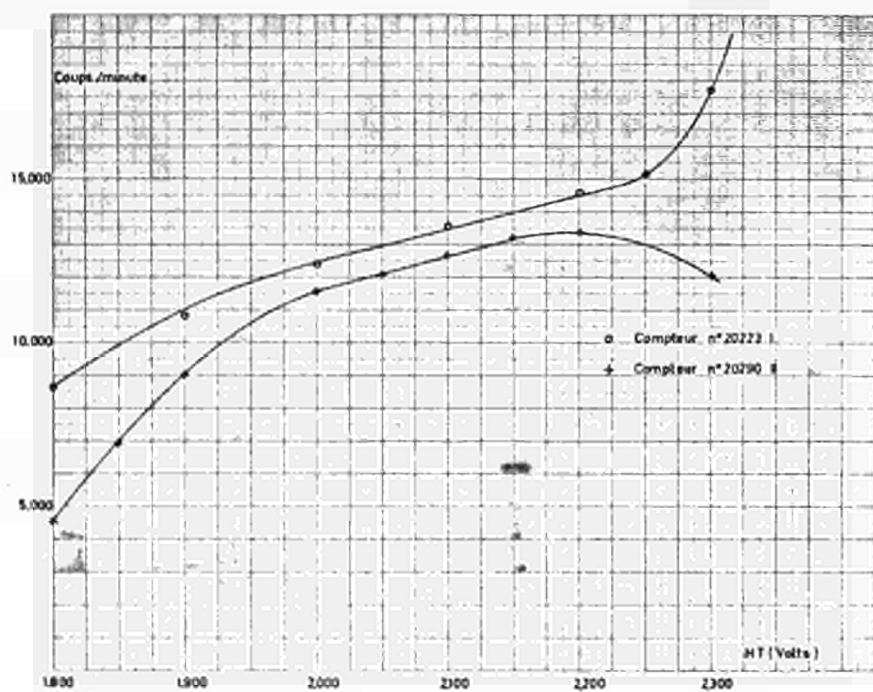


FIGURE 2

Titre : Détermination des courbes de réponse.

Noms des chercheurs : J. CAIZERGUES, Mme M. DOLIAS, M. BRICKA

Description des résultats :

Le calcul des réponses des sphères de Bonner utilisant la théorie du transport multigroupe avait été entrepris dans le cadre du groupe de travail inter SPR N° 8 (Dosimétrie des neutrons) (1).

Les données de ces calculs ont été confrontés avec les résultats des mesures en neutrons monoénergétiques faites en 1971 (2).

La matrice des réponses qui a été retenue (figure 3) est obtenue par recalage sur les valeurs expérimentales des courbes données par le calcul.

Des mesures ont été entreprises pour déterminer les réponses au voisinage de 0,4 eV. On utilise, selon une méthode signalée par HUGUES, deux épaisseurs différentes de cadmium - 0,2 mm et 1,4 mm - La différence entre les comptages obtenus sous ces deux écrans de cadmium constitue la réponse à des neutrons d'énergie moyenne 0,4 eV.

La difficulté réside dans la mesure correcte du flux de neutrons dans cette "fenêtre".

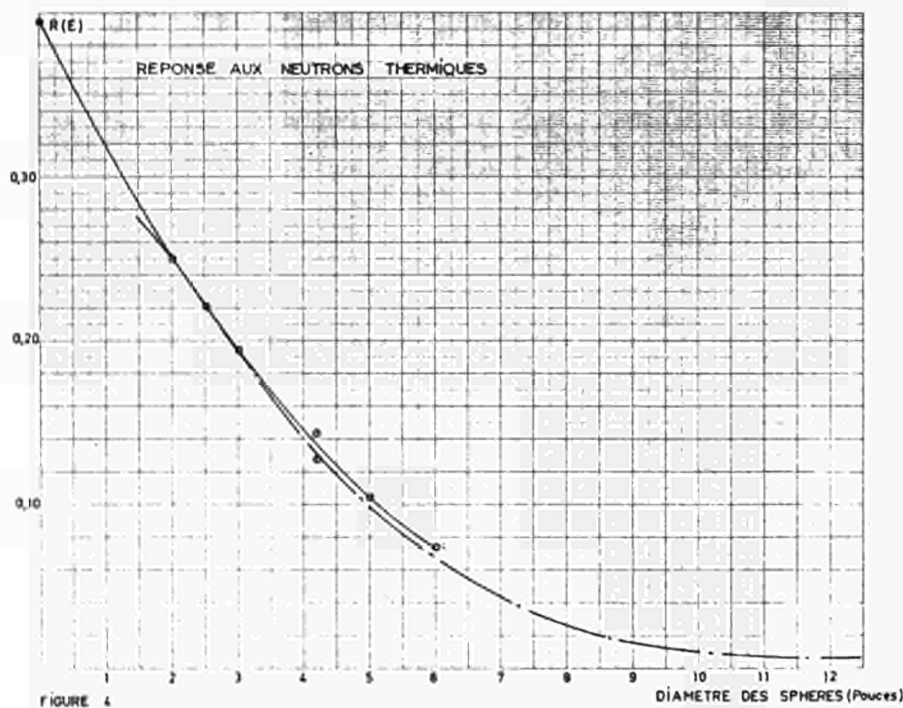
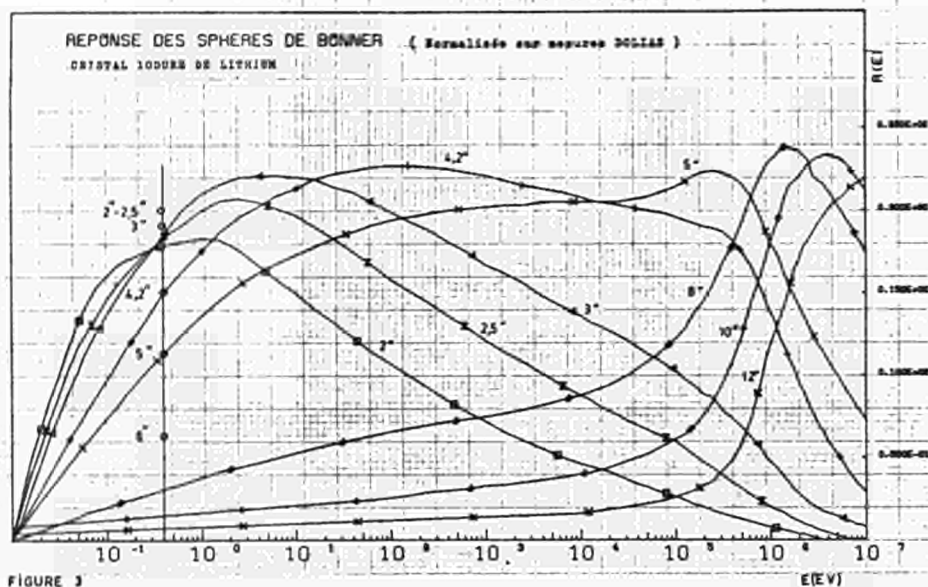
Une première mesure a été effectuée avec le cristal d'iodure de lithium, considéré comme un détecteur noir pour l'énergie 0,4 eV. Les points expérimentaux obtenus par cette méthode sont reportés sur la figure 3.

La réponse aux neutrons thermiques a été mesurée pour les sphères équipées de compteurs à hélium (figure 4).

La courbe de réponse calculée a été recalée sur les résultats expérimentaux, le calcul ne permettant pas d'atteindre directement le rendement du compteur à hélium. L'accord entre le calcul et l'expérience peut être considéré comme satisfaisant.

(2) Mme M. DOLIAS
Rapport EURATOM N° 4791 F (1972)

(1) J. CAIZERGUES - G. POUILLOT
Rapport CEA N° 4400



Titre : Spectrométrie par la technique multisphère.

Noms des chercheurs : M. BRICKA, J. CERCY.

Description des résultats :

Un premier essai utilisant des données de mesure antérieures avait pour but de vérifier la validité de la matrice des réponses. Il mettait en oeuvre un ensemble de détecteurs à activation et des sphères de différents diamètres. Les spectres obtenus sont présentés sur la figure 5.

Le tableau I donne les écarts entre valeurs mesurées et valeurs recalculées à partir des spectres.

Il apparaît une divergence systématique entre le soufre et les sphères de 10" et 12" qui couvrent la même bande d'énergie. Une intercalibration de ces différents détecteurs permettrait de réduire les écarts.

Compte tenu des conditions dans lesquelles ont été étalonnées les sphères, d'une part, les détecteurs de soufre d'autre part, on peut estimer que c'est l'étalonnage de ces derniers qui est à revoir. La source Silène, actuellement en cours de réalisation et qui pourra fonctionner à des niveaux de puissance très variés, permettra l'intercalibration des deux systèmes de détecteurs en dépit de la grande différence de sensibilité.

Une spectrométrie multisphère a été également faite pour le convertisseur sous écran. La figure 6 donne le spectre obtenu qui peut être comparé au spectre correspondant déterminé à l'aide d'un compteur proportionnel à protons de recul (2).

Ce spectre correspond à une bande d'énergie pour laquelle les réponses des sphères ont été mesurées en neutrons monoénergétiques, donc peuvent être considérées comme convenablement connues. La méthode des spectres modèles, mise en oeuvre pour l'interprétation des données de mesure (programme de calcul SESR 1590) permet, semble-t-il, une spectrométrie suffisamment correcte

(2) G. BENEZECH, M. BROSSON, R. VENTRE.

EURATOM - Rapport Annuel 1971 - Programme Biologie - Protection Sanitaire
pages 192 - 193.

TABLEAU I

ECARTS ENTRE LES REPONSES MEASUREES ET LES REPONSES RECALCULEES

Détecteurs	HPRR Nu		HPRR Ecran acier		Castor 0,82 m		Castor 3,26 m	
	$\int_{10^{-16}}^{\sigma(E) \varphi(E) dE}$ neutrons $\int_{10^{11}}^{R(E) \varphi(E) dE}$ impulsions	Ecart %	$\int_{10^{-16}}^{\sigma(E) \varphi(E) dE}$ neutrons $\int_{10^{11}}^{R(E) \varphi(E) dE}$ impulsions	Ecart %	$\int_{10^{-16}}^{\sigma(E) \varphi(E) dE}$ neutrons $\int_{10^{11}}^{R(E) \varphi(E) dE}$ impulsions	Ecart %	$\int_{10^{-16}}^{\sigma(E) \varphi(E) dE}$ neutrons $\int_{10^{11}}^{R(E) \varphi(E) dE}$ impulsions	Ecart %
Cuivre nu	3 460	-0,09	2 080	-0,76	12 400	0,37	2 870	0,24
Or/cadmium	299 000	-0,55	172 000	-0,56	990 000	-1,29	189 000	-1,42
Cuivre/cadmium	1 590	3,26	889	5,33	2 950	6,95	493	6,05
Soufre	842	-6,87	124	-2,21	888	-9,00	63,6	-4,18
Magnésium	12,5	0,50	1,8	0,16	15,8	0,80	1,35	0,43
2" nue	0,667	0,32	0,397	4,00	1,34	-1,70	0,256	-1,03
2"/cadmium	0,559	2,87	0,359	-0,86	0,880	1,78	0,152	0,32
2,5"/cadmium	1,143	-3,78	0,733	-6,11	1,38	-0,36	0,226	0,14
3" /cadmium	1,97	-2,96	1,266	-5,37	1,98	-2,77	0,309	-1,99
4,2"/cadmium	4,12	-2,71	2,58	-3,98	3,05	-2,46	0,438	-2,79
5"	5,19	-6,73	3,11	-6,27	3,47	-6,47	0,445	-2,09
8"	4,98	-2,01	2,68	-2,59	3,02	-3,12	0,343	-5,61
10"	3,54	7,02	1,684	8,94	2,20	6,94	0,226	3,50
12"	2,30	8,97	1,018	7,18	1,56	7,29	0,145	6,35
Fluences	$2,74 \cdot 10^{12}$ n cm ⁻²		$1,53 \cdot 10^{12}$ n cm ⁻²		$2,23 \cdot 10^{12}$ n cm ⁻²		$3,22 \cdot 10^{11}$ n cm ²	

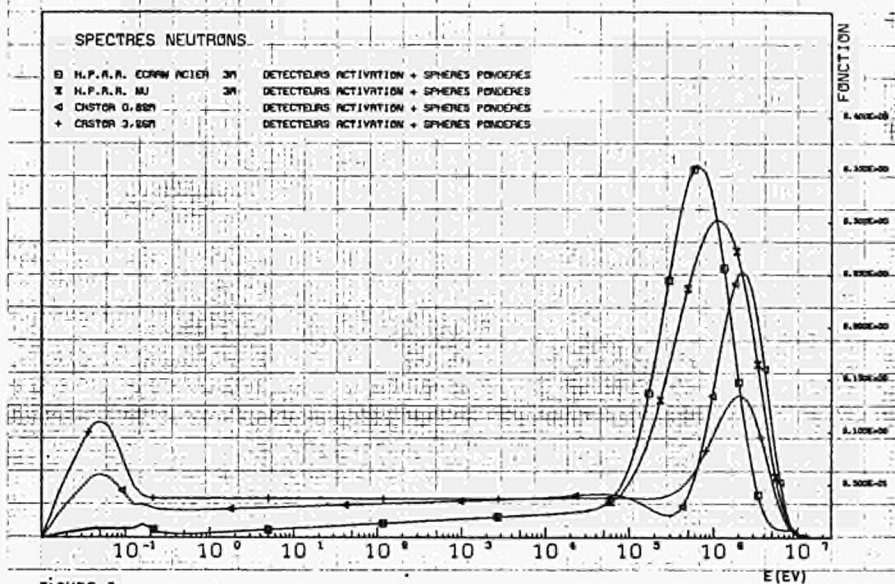


FIGURE 5

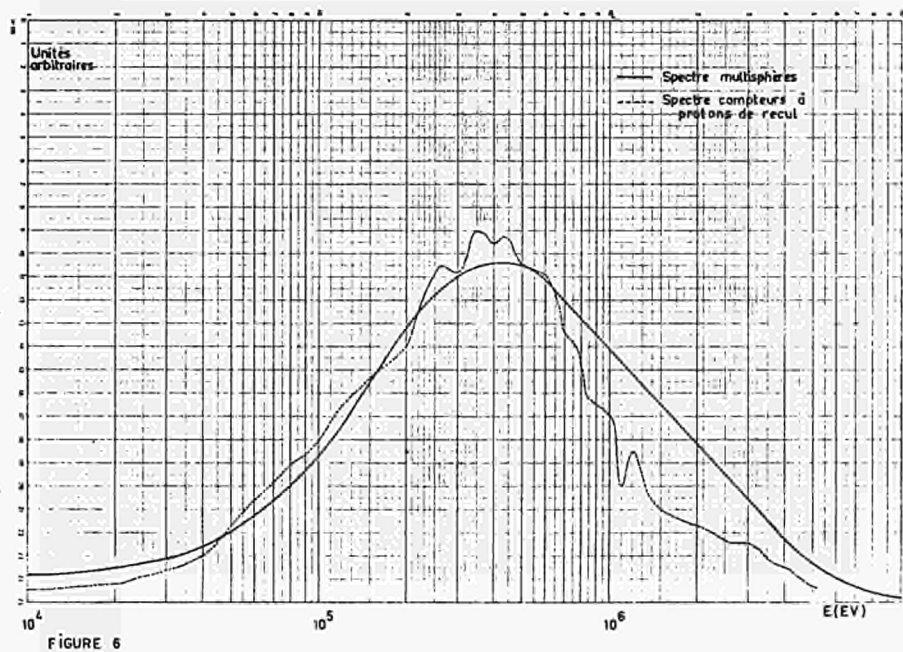


FIGURE 6

Kernforschungsanlage Jülich GmbH
D-517 Jülich, Postfach 365

Nr. des Vertrages: o65-72-1 PSTC

M. Heinzelmann

Neutronendosimetrie mit Spaltfragment-
dosimetern und Kugelmoderator-
dosimetern

Das Projekt befaßt sich mit der Neutronen Orts- und Personendosimetrie. Als Ortsdosimeter wurden Detektoren mit Kugelmoderatoren (Dosimeter nach Bonner) untersucht. Der Einfluß der Detektorgröße im Moderatormittelpunkt wurde berechnet. Weiter wurde der Einfluß des Lichtleiters auf die Empfindlichkeit gemessen für verschiedene Kugeldurchmesser, und es wurde ein unerwartet hoher Lichtleitereinfluß gefunden. Als Personendosimeter wurden die im Vorjahr hergestellten Spaltfragmentdosimeter im praktischen Einsatz geprüft und mit Filmdosimetern verglichen.

Ergebnisse des Projekts Nr. 1

F. Rohloff, M. Heinzelmann

Neutronendosimetrie mit Spaltfragmentdosimetern
und Kugelmoderator dosimetern

a) Untersuchung der Dosimeter mit Kugelmoderator

In diesem Jahre lag das Schwergewicht der Arbeiten auf dem Einfluß des Detektors im Moderatormittelpunkt und der Detektorzuleitungen auf die Empfindlichkeit. Es wurde für 2 verschiedene Moderatorkugeldurchmesser die Empfindlichkeit als Funktion der Neutronenenergie für 4 sehr unterschiedliche Detektoren im Kugelmittelpunkt berechnet. Als Moderatorkugeln wurde eine große und eine kleine Kugel benutzt (10" und 3" Durchmesser). Detektoren im Kugelmittelpunkt sind ein ^3He -Zählrohr und drei LiJ-Szintillatoren mit ganz unterschiedlichem ^6Li -Gehalt.

Gerechnet wurde mit einer "discrete ordinate" Methode. Als Ergebnis erhält man bei einem Moderatorkugeldurchmesser für alle 4 Detektoren, abgesehen von einem Proportionalitätsfaktor, praktisch die gleiche Energieabhängigkeit der Empfindlichkeit. Figur 1 zeigt die Ergebnisse für die 10" Moderatorkugel. Die berechneten Empfindlichkeiten unterscheiden sich erheblich von den Rechnungen anderer Autoren (z.B. Hansen und Sandmeier). Die 3" Moderatorkugel zeigt nach den neueren Rechnungen eine geringere Energieabhängigkeit und bei der 10" Kugel ist im Bereich von 10^5 bis 10^6 eV der Verlauf steiler. Eine der nächsten Aufgaben wird sein zu prüfen, warum die neueren Ergebnisse von den alten Rechnungen sich unterscheiden.

Da bei Messungen mit Detektoren nach Bonner verschiedene Arbeitsgruppen ganz verschiedene Detektorzuführungen verwenden, wurde geprüft, wie weit die Detektorzuführung die Empfindlichkeit beeinflusst.

Es wurden Indiumfolien im Mittelpunkt von Moderator-
kugeln mit den Neutronen einer Am-Be-Quelle bestrahlt.
Die Indiumfolie saß entweder an der Spitze eines Licht-
leiters oder in einer homogenen Polyäthylenkugel. Die
Messungen wurden mit Quarz- und Plexiglaslichtleitern
von 12,7 und 15,8 mm Durchmesser ausgeführt. Der Ein-
fluß des Lichtleiters ist vor allem bei kleinen
Moderatorkugeln erheblich, und er darf nicht vernach-
lässigt werden. Die verschiedenen Mehr-Kugel-Verfahren
in der Neutronendosimetrie sind aufgrund dieser Ergeb-
nisse neu zu überprüfen. Der dünnere Quarzlichtleiter
verringerte die Flußdichte thermischer Neutronen im
Mittelpunkt der 2" Kugel um 28 %. Eine Aluminiumhülle
von 1 mm Dicke um den Lichtleiter verringert die Fluß-
dichte im Kugelmittelpunkt weiter um ca. 14 %.(Figur 2)
Für eine dicke Aluminiumhülle wurde gezeigt, daß der
Lichtleitereinfluß energieabhängig ist. Die Flußdichte
thermischer Neutronen wird bei Bestrahlung mit den
Neutronen einer Am-Be-Quelle um 15 % mehr verringert
als mit den Neutronen einer Am-Li-Quelle. Diese Messungen
sollen mit weiteren Detektorzuführungen fortgesetzt
werden.

b) Spaltfragmentdosimetrie

Von den im vorigen Jahr hergestellten Spaltfragment-
dosimetern wurden 10 monatlich in Jülich von Personen
getragen, die viel in Strahlenfeldern mit Neutronen
arbeiten. 2 weitere Dosimeter wurden an Personen ausge-
geben, die mit Sicherheit nicht in Neutronenfeldern
arbeiten. Die gemessenen Neutronendosen waren gering.
Bei den 2 Kontrollpersonen wurden nicht mehr als
2 Spuren/Monat, im Mittel aber weniger als 1 Spur/Monat
festgestellt. 2 Spuren entspricht 10 mrem. Auf den
anderen Dosimetern wurden häufig Dosisbelastungen von
20 bis 35 mrem/Monat gemessen. Der Maximalwert betrug
50 mrem/Monat.

Die höheren Werte hätten eigentlich gerade mit den Kernspurfilmern meßbar sein müssen, insbesondere wenn man berücksichtigt, daß die Kernspurfilmern für Neutronen mit Energien ab 500 keV, die Spaltfragmentdosimeter aber erst ab 1,3 MeV empfindlich sind. Diese Ergebnisse machen aber auch verständlich, warum in Jülich nie eine Dosisbelastung auf Kernspurfilmern gefunden wurde.

c) Zusammenarbeit mit Herrn Bricka, Cadarache

Durch denselben Vertrag werden auch Arbeiten mit Detektoren nach Bonner in Cadarache gefördert. Zur Koordinierung dieser Arbeiten war im Mai 1972 eine Besprechung in Cadarache mit den Herren Bricka, Caizergues und Lamberieux.

d) Veröffentlichungen und Vorträge

M. Heinzelmann, F. Rohloff, H. Schüren,
Einfluß gestreuter Neutronen auf die Kalibrierung von
Detektoren nach Bonner
Atomkernenergie 19 (1972) 312.

F. Rohloff,

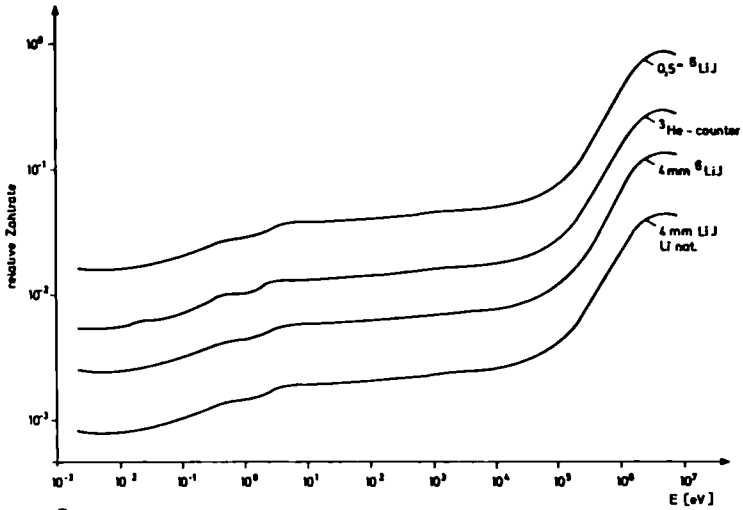
Spezielle Probleme bei der Neutronendosismessung mit
Moderator-Detektor-Systemen
Proceedings First Symposium on Neutron Dosimetry in
Biology and Medicine, EUR 4896 (1972) p. 405.

M. Heinzelmann, H. Schüren,
Thoriumspaltfragmentdosimeter in der Neutronendosimetrie
Proceedings First Symposium on Neutron Dosimetry in
Biology and Medicine, EUR 4896 (1972) p. 315.

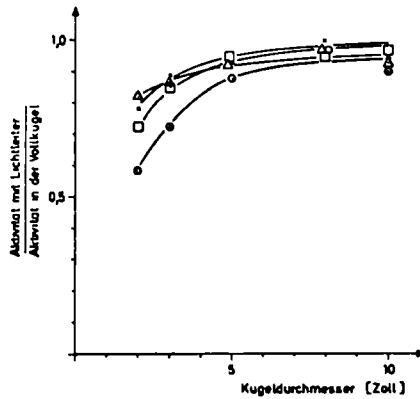
F. Rohloff, M. Heinzelmann,

The Influence of Detector-type and -equipment on the
Sensitivity of Bonner Spheres

IAEA Symposium on Neutron Monitoring for Radiation
Protection Purposes, Vienna 1972.



Figur 1: Empfindlichkeit einer Moderatorkugel nach Bonner von 10" Durchmesser mit verschiedenen Detektoren im Kugelmittelpunkt.



Figur 2: Einfluß eines Quarzlichtleiters und einer Aluminiumhülle um den Lichtleiter auf die Empfindlichkeit von Detektoren nach Bonner

Durchmesser des Lichtleiters: 12,7 mm

Dicke der Aluminiumhülle: 1 mm

Einfluß des Lichtleiters allein:

- thermische Neutronen
- × epithermische Neutronen

Einfluß des Lichtleiters mit Aluminiumhülle:

- thermische Neutronen
- △ epithermische Neutronen

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

Nr. des Vertrags: 065 - 72 - 1 PSTC

Leiter der Forschungsgruppe(n): Prof. Wachsmann u. Dr. G. Burger

**Allgemeines Thema des Vertrags:
Strahlenschutz - Personendosimetrie**

Gegenstand des Vorhabens ist sowohl die Verbesserung der Neutronendosimetrie im Strahlenschutz als auch der damit zusammenhängenden Kalibriertechnik. Verbesserungen in der Personendosimetrie sind derzeit nur in zwei Richtungen zu erwarten, nämlich durch die Einführung der nichtphotographischen Kernspurtechnik und durch die Verwendung von Albedo-Dosimetern zur Erweiterung des erfassbaren Energiebereichs. Für den Nachweis schneller Neutronen scheint sich Np-237 durchzusetzen. Die Arbeiten daran wurden mangels geeigneter Konverter unterbrochen und werden 1973 unter Verwendung von Np-Aluminium-Folien fortgesetzt. Zwischenzeitlich werden besonders die Auswertverfahren verbessert. Die theoretischen Grundlagen für die Albedotechnik wurden mit Hilfe der weiterentwickelten Neutronentransportprogramme in Angriff genommen.

Da Moderator-detektoren in der Strahlenschutzdosimetrie eine wesentliche Rolle spielen /4/, wurden deren Eigenschaften theoretisch und experimentell weiter untersucht.

Der Schwerpunkt der Arbeiten lag auf dem Gebiete der Kalibriertechnik. Hier wurden die Arbeiten zur Neutronenspektrometrie fortgesetzt, sowohl in Richtung der Verbesserung der experimentellen Methoden als auch der numerischen Datenanalyse. (Spektrententfaltung)

Im Rahmen eines Vergleichsprogrammes der Gemeinschaft wurden Radionuklidquellen kalibriert. Die Quellstärkebestimmung erfolgt dabei mit dem MnSO_4 -Bad und dem Precision Long Counter, die Spektrometrie mit dem Szintillationspektrometer.

Ergebnisse des Projekts Nr. 1.

Leiter des Projekts und wissenschaftliche Mitarbeiter:
H. Schraube, W. Abmayr, F. Grünauer, K. Kolbe

Titel des Projekts:
Neutronendosimetrie und Kalibriertechnik

Ein Hauptziel war die automatische Auswertung von Ätzspuren. Unter Einbeziehung der Katastrophen-Dosimetrie soll ein Spurdichtebereich von $1 - 10^5$ Sp/cm² überstrichen werden können. Bis $4 \cdot 10^2$ Sp/cm² verwenden wir einen Funkenzähler nach Cross und Tommasino. Für die Auswertung hoher Spurdichten werden lichtoptische Methoden eingesetzt, nämlich die Abtastung der Detektorfolien durch ein Flying-Spot Mikroskop, das zur schnellen Datenverarbeitung online an einen Computer angeschlossen ist und durch ein kommerzielles Fernseh-Mikrobildabtastgerät. Mit geeigneten Verfahren können Spur-Überlappungen korrigiert und Spurdichten bis zu $4 \cdot 10^5$ Sp/cm² mit guter Linearität nachgewiesen werden.

Zur rechnerischen Bestimmung der Empfindlichkeitsfunktionen von Moderator-detektoren bieten sich bekannte SN-Codes an. Der zweidimensionale Code DØT-2 sowie der eindimensionale Code ANISN wurden an eine Rechenanlage 360 -91 angepaßt und an einer Reihe von "benchmark"-Problemen getestet. Für den Code ANISN wurde auch die adjungierte Rechnung durchgeführt und am Beispiel der Bonner Moderator-kugeln erprobt. Sowohl die Bonner Kugeln als auch der de Pangher Precision Long Counter wurden in streustrahlarmer Geometrie in der Neutronenmeßhalle der GSF Neuherberg mit Radionuklidquellen kalibriert und die Ergebnisse der Kugeln mit denen der Rechnungen verglichen. An Hand der Ergebnisse wurden Kalibriervorschriften für Moderator-detektoren erarbeitet /3/.

Die Rechenergebnisse für die Empfindlichkeitsfunktionen des Teleskop-Spektrometers wurden mit monochromatischen 14 MeV-Neutronen aus der D, T-Reaktion an einem 400 kV-Van De Graaff-Generator unter Verwendung der associated-particle Methode absolut überprüft. Für das Szintilla-

tionsspektrometer erfolgt die Auswertung der Impulshöhenverteilung nach zwei Verfahren, dem vereinfachten sogenannten Differentiationsverfahren und einem Entfaltungsverfahren unter Berücksichtigung der realistischen Empfindlichkeitsfunktionen. Die früher vorhandenen größeren Diskrepanzen zwischen den beiden Verfahren sind z. T. erklärt und beseitigt /4/. Bis auf noch ungeklärte kleinere Unstimmigkeiten in der Energieeichung zeigen besonders die nach der vereinfachten Auswertemethode gewonnenen Ergebnisse des Szintillationsspektrometers eine gute Übereinstimmung mit denen des Teleskop-Spektrometers.

Die für die verschiedenen Anwendungsbereiche im Rahmen der früheren Verträge entwickelten Methoden der Spektrenentfaltung wurden einer zusammenfassenden kritischen Würdigung unterzogen. Dazu wurden die gebräuchlichsten aus der Literatur bekannten Verfahren hinsichtlich ihrer mathematischen Merkmale klassifiziert und mit den eigenen Verfahren verglichen /5/.

Literatur

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Automatic Measurements of Tracks in Solid State Track Detectors
Proceedings 8th Intern. Coll. on Corp. Photogr. Bukarest, 1972
- /2/ D. Nachtigall and G. Burger
Dose Equivalent Determinations in Neutron Fields
in Topics in Radiation Dosimetry, Academic Press, 1972
- /3/ H. Schraube, F. Grünauer, and G. Burger
Problems in calibration of neutron moderator detectors
IAEA/SM-167/32, Vienna, 1972
- /4/ H. Schraube, W. Eckl, K. Kolbe and G. Burger
The Spectrometry of Fast Neutrons with Different Methods
Proc. First Symp. on Neutron Dos. Biol. Med. EUR 4896 d-f-e, 1972
- /5/ F. Grünauer and W. Schmatz
Data analysis for low resolution neutron spectrometry
IAEA/SM-167/31, Vienna, 1972

Vertragspartner der Kommission: Bundesforschungsanstalt
für Lebensmittelfrischhaltung, Karlsruhe

Nr. des Vertrags: 047-69-4 PST C

Leiter der Forschungsgruppe: Prof. Dr. J.F. Diehl

Allgemeines Thema des Vertrags: Meßbare Veränderungen in
bestrahlten Lebensmitteln

In den vergangenen Jahren sind in mehreren Ländern Genehmigungen für die Behandlung von Lebensmitteln mit ionisierenden Strahlen erteilt worden. Eine EWG-Richtlinie für die Zulassung der Kartoffelbestrahlung in den Mitgliedsländern der Europ. Gemeinschaft ist in Vorbereitung. Den mit der Lebensmittelüberwachung beauftragten Stellen steht jedoch bis heute keine allgemein anerkannte Methode zur Verfügung, die eine Unterscheidung bestrahlter von unbestrahlten Lebensmitteln gestattet. Das Interesse an der Entwicklung solcher Methoden ist daher groß.

Abgesehen vom Problem der Überwachung interessiert die Untersuchung der durch die Bestrahlung in Lebensmitteln verursachten chemischen und physikalischen Veränderungen auch im Hinblick auf toxikologische und technologische Fragen. Ziel der in Teil B beschriebenen Arbeiten ist einerseits die Prüfung verschiedener analytischer Methoden auf ihre Eignung zum Nachweis einer erfolgten Bestrahlung von Lebensmitteln und andererseits die Vertiefung der Kenntnisse über die strahleninduzierten Veränderungen in Lebensmitteln.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts: Dr. H. Scherz, Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe

Titel: Elektrische Leitfähigkeit

Frühere Ergebnisse hatten gezeigt, daß bei Kartoffeln die elektrische Leitfähigkeit durch die Bestrahlung erniedrigt wird. Die Abhängigkeit der erhaltenen Werte von der Strahlendosis ist nicht linear; nach einem

anfänglich starken Abfall wird bei höheren Dosen ein konstanter Endwert erreicht. Die Untersuchungen wurden auf andere Materialien ausgedehnt: Bei Bestrahlung von Äpfeln, Karotten, Zwiebeln, Futterrüben, Kohlrabi und Spargel (Dosis: 10, 20, 50 und 100 krad) konnte keine signifikante Änderung der Leitfähigkeit festgestellt werden.

Bei unbestrahlten Kartoffeln war ferner beobachtet worden, daß unmittelbar nach dem Einstechen der Elektroden die Leitfähigkeit abfiel und nach drei Minuten einen konstanten Wert erreichte, der 20 - 30 % unter dem Anfangswert lag. Dieser Leitfähigkeitsabfall wird durch die Bestrahlung stark reduziert und ab 50 krad erhält man unmittelbar nach dem Einstechen der Elektroden den Endwert. Da dieser Effekt Verminderung des Leitfähigkeitsabfalls von der Sorte weitgehend unabhängig ist, kann er als ein Unterscheidungsmerkmal zwischen bestrahlten und unbestrahlten Kartoffeln gelten. Mit Sicherheit lassen sich Strahlendosen größer als 10 krad über einen Lagerzeitraum von 4 Monaten nachweisen. Der Leitfähigkeitsabfall nach dem Einstechen der Elektroden konnte auch bei Rüben, Kohlrabi und Spargel festgestellt werden. Allerdings ist der Effekt geringer als bei Kartoffeln und zeigt keine Korrelation mit den angewandten Strahlendosen.

Veröffentlichungen:

H. Scherz: Conductivity measurements as a method for differentiation between irradiated and nonirradiated potatoes.
EURATOM Bericht, im Druck

Ergebnisse des Projekts Nr. 2

Leiter des Projekts: Dr. H. Scherz, Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe

Titel: Chromatographie und Kolorimetrie

Bei der Untersuchung der radiolytischen Spaltprodukte von Kohlenhydraten wurden Malondialdehyd, Desoxy- bzw. Desoxycarbonylverbindungen und 1,4-Pyronverbindungen gefunden. Da diese Substanzen bei der Bestrahlung in

Lebensmitteln nur in sehr geringen Mengen gebildet werden, ist es notwendig, zu deren Nachweis und quantitativer Bestimmung geeignete Anreicherungsverfahren auszuarbeiten. Als Untersuchungsobjekt diente Weizenmehl.

Durch fest-flüssig Extraktion mit dem Lösungsmittelgemisch Äthylacetat-Aceton-Wasser 4:5:1 (v/v) wurden neben geringen Mengen an strahleninduzierten Spaltprodukten große Mengen an Lipiden und Phosphatiden mit extrahiert, die im weiteren Verlauf der Aufarbeitung Emulsionen bildeten, die eine quantitative Isolierung unmöglich machten.

Bessere Ergebnisse wurden durch eine Kombination von Dialyse mit flüssig-flüssig Extraktion erzielt. Aufschlämmungen von bestrahlten Mehlproben wurden in Dialysierschläuche gefüllt und diese in die wässrige Phase der Extraktoren eingebracht. Die niedermolekularen Spaltprodukte diffundieren durch die Schlauchwände, und die mehr hydrophoben Substanzen werden kontinuierlich in die organische Phase überführt. Nach deren Abdampfen konnten die 1,4-Pyrone nach dünn-schichtchromatographischer Trennung in bestrahltem Mehl ab 2 Mrad einwandfrei nachgewiesen werden.

Veröffentlichungen:

- 1) H. Scherz: Über die Bildung von Malondialdehyd bei der Bestrahlung von Lebensmitteln.
Chem. Mikrobiol. Technol. Lebensm. 1 (1972) 103
- 2) H. Scherz: Die Bildung von 1,4-Pyronen bei der Radiolyse von Stärke.
Z. Naturforschg., im Druck

Ergebnisse des Projekts Nr. 3

Leiter des Projekts: Dr. B.J. Radola, Bundesforschungsanstalt für Lebensmittelfrischhaltung, Karlsruhe

Titel: Chromatographie, isoelektrische Fokussierung und Enzymanalyse

In Fleisch, das mit einer Dosis von 1 bzw. 5 Mrad bestrahlt wurde, lassen sich mit Hilfe verschiedener Enzymnachweise in den Sarkoplasma-Proteinen strahleninduzierte Veränderungen feststellen, die es ermöglichen,

eine erfolgte Bestrahlung zu erkennen und die angewandte Dosis abzuschätzen. Bei der Dünnschicht-Gelfiltration wurde bei Anwendung chromogener und fluorogener Nachweisverfahren für Esterasen und Lipasen zusätzlich zu den 2-3 im unbehandelten Fleisch vorhandenen enzymatisch aktiven Zonen eine strahleninduzierte Fraktion gefunden, die aufgrund des hohen R_M Wertes von $\sim 2,4$ leicht zu erkennen war. Diese Fraktion hatte denselben R_M Wert wie eine bereits früher bei der Proteinfärbung gefundene strahleninduzierte Fraktion. Empfindliche Sekundärsubstrate ermöglichten auch den Nachweis einer strahleninduzierten Fraktion mit Peroxidaseaktivität. Der R_M Wert dieser Fraktion lag ebenfalls bei $\sim 2,4$. In Modellversuchen wurde ein Gemisch aus Sarkoplasma-Proteinen aus Rindfleisch und einer vorgereinigten Meerrettich-Peroxidase bestrahlt. Bei steigender Strahlendosis traten zunehmende Mengen enzymatisch aktiver Aggregate auf, die bei der Dünnschicht-Gelfiltration mit einem R_M Wert von $\sim 2,4$ wanderten. Aufgrund dieser Modellversuche ist es sehr wahrscheinlich, daß es sich auch bei der im bestrahlten Fleisch gefundenen Fraktion mit $R_M \sim 2,4$ um strahleninduzierte enzymatisch aktive Aggregate handelt. Verglichen mit den bisher angewandten Methoden der Proteinfärbung sind die enzymatischen Nachweismethoden einer erfolgten Bestrahlung einfacher und schneller.

Bei der dünn-schicht-isoelektrischen Fokussierung und der Disk-Fokussierung wurden durch enzymatische Nachweisverfahren charakteristische Änderungen des Fokussierungsmusters festgestellt, die einen zusätzlichen Hinweis auf eine erfolgte Bestrahlung ermöglichen. Es tritt eine dosisabhängige Verminderung der basischen Proteine sowie einiger Komponenten im Bereich pH 6-7 ein. Auch bei Enzymen (Esterasen, Peroxidasen) wurden bei der Bestrahlung die basischen Isoenzyme bevorzugt inaktiviert. Die strahleninduzierten Veränderungen des Fokussierungsmusters der Proteine sind nach Bestrahlung mit 5 Mrad gut sichtbar. Charakteristisch für das Muster der Proteine aus bestrahltem Fleisch sind - neben quantitativen Unterschieden - unscharfe Zonen und eine starke Untergrundfärbung zwischen den Protein-zonen.

Ergebnisse des Projekts Nr. 4

Leiter des Projekts und wissenschaftliche Mitarbeiter:
Prof. Dr. F. Drawert, Dr. B. Beck, Institut für chemisch-
technische Analyse und chemische Lebensmitteltechnologie
der Technischen Universität München

Titel: Gaschromatographie und Massenspektrometrie

Tripalmitat, Tristearat, Ölsäuremethylester, Linolsäuremethylester, Laurinsäure, Schweinefett, Palmin, Sonnenblumenöl und Olivenöl wurden mit einer Dosis von 0.5 bis 6 Mrad bestrahlt. Nach Trennung der Kohlenwasserstoffe von den Sauerstoffverbindungen an Kieselgel unter Verwendung von Pentan bzw. Pentan/Äther als elutrope Reihe wurden in den mit 6 Mrad bestrahlten Proben 28 Kohlenwasserstoffe und 24 Sauerstoffverbindungen gaschromatographisch getrennt und massenspektrometrisch identifiziert.

Aufgrund der gefundenen Spaltprodukte sind anhand der Literatur Radikalmechanismen zu diskutieren, was auch Veranlassung dazu gab, Fette nach 24stündigem Erhitzen auf 170° C zu untersuchen. Auch hier konnten 28 Kohlenwasserstoffe identifiziert werden, die aber zusätzlich Cyklohexene enthalten, wodurch eine Unterscheidung zwischen bestrahlten und erhitzten Fetten möglich ist. Unter den gewählten Bedingungen werden die erhitzten Fette stärker verändert als die bestrahlten.

Veröffentlichungen:

- 1) F. Drawert, B. Beck: Bildung von 9-Oxo-nonansäuremethylester und Nonanal aus Ölsäuremethylester von 9-Oxo-nonansäure aus Schweinefette durch Bestrahlung mit 6 Mrad.
Chem. Mikrobiol. Technol. Lebensm. 1 (1972) 110 - 111
- 2) F. Drawert, B. Beck: Bildung von 9-Oxo-nonansäuremethylester und 12-Oxo-dodecesäuremethylester aus Linol- und Linolensäuremethylester und von 9-Oxo-nonansäure aus Sonnenblumenöl durch Bestrahlung mit 6 Mrad.
Chem. Mikrobiol. Technol. Lebensm. 1 (1972) 158 - 159

Ergebnisse des Projekts Nr. 5

Leiter des Projekts und wissenschaftliche Mitarbeiter:
Dr. H. Penner, S. Shirsat, Bundesforschungsanstalt für
Lebensmittelfrischhaltung, Karlsruhe

Titel: Mikrostruktur, Chromatographie

Zur Beantwortung der Frage, ob früher mit histologischen Methoden festgestellte Strahlenwirkungen auf die Wundheilung auch mit biochemischen Methoden nachweisbar sind, wurde der Einfluß einer Bestrahlung (Dosis 10 - 15 krad) auf stoffwechselphysiologische Vorgänge in Kartoffelknollen untersucht. Gemessen wurden die Aktivität der Phenylalaninammonialyase (PAL) und der Gehalt an Chlorogensäure in bestrahlten und unbestrahlten Kartoffeln sowie in inkubierten Gewebeschnitten. Zur Bestimmung der Chlorogensäure wurde eine dünnschichtchromatographische Methode mit densitometrischer Auswertung entwickelt.

Werden Kartoffelscheiben oder -würfel in einer feuchten Kammer inkubiert, so nimmt der Gehalt an Chlorogensäure sowie die Aktivität der PAL zu. Bei bestrahlten Kartoffeln zeigt sich mit zunehmender Strahlendosis eine verstärkte Chlorogensäuresynthese und eine abnehmende PAL-Aktivität. Diese Effekte sind jedoch stark von der Kartoffelsorte und von Lagerungsbedingungen abhängig und daher für eine Identifizierung bestrahlter Kartoffeln nur beschränkt geeignet.

Veröffentlichungen:

- 1) H. Penner und H. Fromm: Versuche zum Nachweis einer erfolgten Bestrahlung von Kartoffeln. II. Bestimmung der Chlorogensäure. Z. Lebensmittel-Unters. -Forschg. 150 (1972) 84
- 2) H. Penner: Versuche zum Nachweis einer erfolgten Bestrahlung von Kartoffeln. III. Die Wirkung von Röntgenstrahlen auf die Chlorogensäurebiosynthese in der Kartoffelknolle. Potato Research, im Druck
- 3) S. Shirsat und H. Penner: Versuche zum Nachweis einer erfolgten Bestrahlung von Kartoffeln. IV. Die Wirkung von Röntgenstrahlen auf die Bildung von Phenylalaninammonialyase in Kartoffeln. Z. Lebensmittel-Unters. -Forschg., im Druck

Results of the project Nr. 047-69-4 PSTC

Head of the team / scientific coworkers: L.Strackee

D.Onderdelinden

Title of the project: Detection of irradiated foodstuffs by
means of electron spin resonance

Description of the results obtained in the period
Jan 1 - Dec 31 1972

The investigations performed during the above mentioned period were mainly concentrated on ESR measurements of irradiated plastic materials. The materials were obtained from commercial sources and were used without further purification and are listed below.

1. polyethene a) high pressure polyethene
 b) low pressure polyethene, low molecular weight
 c) low pressure polyethene, high molecular weight
2. polypropene
3. polystyrene
4. polyacetale
5. polytetrafluorethene
6. polycaprolactam
7. polyvinylchloride a) density 1.39 gram cm⁻³
 b) density 1.35 gram cm⁻³
8. polymethylmethacrylaat
9. phenolformaldehyde paper
10. phenolformaldehyde tissue
11. polycarbonate

The formation and decay of radicals induced in these materials by γ rays was studied. An evaluation of the ESR spectra measured after the irradiation leads to identification of the radicals in most cases. The decay behaviour of these radicals was studied quite extensively.

Quite generally it follows from the performed study on the induction of free radicals in polymers that can be used as packaging material that for most cases the radicals can be measured during a period of the order

of 2 months after irradiation with a dose of one Mrad. Exceptions are formed for polymers with a large fraction in the glass state above the glass temperature and for polymers with plasticizers. The number of radicals created in the polymers was found to depend on irradiation conditions such as dose rate, temperature and gas environment. A model with accounts qualitatively for some of these parameters is given. Decay times were also found to depend on these parameters. It is therefore not possible to deduce accurately from an ESR measurement to what dose a sample is irradiated, even if the date of any possible irradiation can be estimated. An exception is formed by Teflon, where at room temperature no decay of any significance could be observed.

The results are presented in a report entitled: "ESR Study on irradiated plastics", which has been accepted for publication as an EUR-report.

- B. At the end of above mentioned period measurements were started on changes in the electrical conductivity of irradiated potatoes. The first measurements were performed with the potato variety "Bintje" and were consistent with the results obtained by dr.Scherz in Karlsruhe.

Contrat n° 047-69-4 PSTC

Institut d'Hygiène et d'Epidémiologie
Bruxelles (Belgique)
Directeur : Prof. Dr. A. LAFONTAINE

Chefs du projet : Dr. L. BUGYAKI
Dr. M. VANDER STICHELEN ROGIER.

1. Application de techniques électrophorétiques à l'identification de l'irradiation de certains aliments.

Deux techniques ont été testées dans ce but :
électrophorèse en gels de polyacrylamide (méthode d'Ornstein et Davis) et focalisation isoélectrique en gels de polyacrylamide (ampholines de pH 3-10; 7-10; 3-6 et 5-8).
Elles ont été appliquées aux extraits protéiniques des aliments irradiés (à la température ordinaire) suivants :

- viande de boeuf (5 Mrad et 0,5 Mrad)
- filet de cabillaud (1 Mrad)
- oeuf entier homogénéisé (1 Mrad)
- crevette grise cuite et décortiquée (200 krad)
- champignon de couche (250 krad)
- oignon (10 krad)
- pomme de terre blanche (15 krad).

Les échantillons irradiés ainsi que les témoins non irradiés ont été conservés en réfrigérateur sauf les oignons et les pommes de terre qui ont été conservés dans une cave. Les protéines ont été extraites au moyen de solutions tampons adéquates. Dans le cas des crevettes, des champignons, des oignons et des pommes de terre, les extraits furent lyophilisés après dialyse contre de l'eau distillée.

Electrophorèse en gels de polyacrylamide.

- protéines sarcoplasmiques de la viande et du poisson : certaines différences apparaissent entre les séparations électrophorétiques des extraits protéiniques des échantillons irradiés et non irradiés, notamment dans l'affaiblissement ou la disparition de certaines zones colorées.
- oeufs : de faibles différences, dues à l'irradiation, apparaissent dans les séparations électrophorétiques des différents extraits. Toutefois, la congélation d'un échantillon modifie plus les séparations électrophorétiques que ne le fait l'irradiation ce qui, par conséquent, rend la méthode inadéquate pour détecter l'irradiation.
- crevette : nous ne sommes pas parvenus à reproduire les résultats d'une méthode d'identification publiée. De nouveaux essais en ce sens seront entrepris.
- champignons, oignons et pommes de terre : il n'apparaît pas de différences notables entre les séparations des extraits protéiniques des échantillons irradiés et non irradiés.

Focalisation isoélectrique

- protéines sarcoplasmiques de la viande et du poisson : certaines différences apparaissent entre échantillons irradiés et non irradiés mais les expériences sont toujours en cours.
- oeufs : mêmes conclusions que pour l'électrophorèse.
- crevettes : les séparations obtenues sont mauvaises. De nouveaux essais seront entrepris avec d'autres tampons d'extraction.
- champignons et oignons : aucune différence notable n'apparaît entre les extraits protéiniques des échantillons irradiés et échantillons témoins.
- oignons : une certaine différence apparaît, provoquée par l'irradiation mais ce résultat doit être confirmé par de nouvelles expériences.

2. Identification des champignons irradiés au moyen de cultures cellulaires.

Différents milieux de culture (à base d'agar) ont étéensemencés au moyen de boutures prélevées sur des champignons irradiés et des champignons frais non irradiés. Après 24 h. d'incubation à 25° C. on observe la prolifération de filaments (hyphes) en plusieurs endroits de la bouture dans le cas du champignon frais non irradié. Par contre dans le cas des champignons irradiés à la dose 250 krad il n'y a pas de formation d'hyphes nouvelles. Cette méthode simple permet donc d'identifier les champignons irradiés. Des études complémentaires vont être effectuées afin de déterminer la sensibilité du phénomène à la dose d'irradiation.

Publications.

- 1/ L. BUGYAKI et M. VANDER STICHELEN ROGIER - Eur. 4878 (en cours d'impression) : Immunoélectrophorèse et électrophorèse verticale en gel d'amidon des protéines de la viande, du poisson et de l'oeuf entier, irradiés aux rayons gamma.
- 2/ L. BUGYAKI et P. HEINEMANN; Atoomkernenenergie, 19, 261 (1972) : Identification des champignons irradiés.

Contractant de la Commission : CENATRA

Numéro du contrat : O47-69-4- PST C

Directeurs des recherches : Prof. Ir. E. Maes

Prof. Ir. A.R. Deschreider.

Sujet général du contrat : La détection de l'irradiation des denrées alimentaires.

Dans le cadre de l'application des rayons gamma en vue de la conservation des aliments, une attention particulière a été donnée à la détection d'un traitement de ce genre. Il s'agit là d'un problème dont les aspects sanitaires et juridiques sont évidents.

Dans l'étude de ce problème il y a d'une part la recherche des constituants des aliments dont les modifications, sous l'action du rayonnement sont susceptibles de servir de critère pour la détection; et d'autre part, le choix des techniques les plus aptes à déceler des modifications très faibles.

Dans le présent contrat de recherche, c'est à CENATRA que furent confiés les moyens d'investigation relevant de la spectrophotométrie, de la spectropolarimétrie et de l'analyse thermodifférentielle.

Pendant l'année 1972 cet institut s'est attaché à l'examen des trois points suivants :

- a) l'étude en infrarouge différentiel des emballages plastiques alimentaires irradiés.
- b) l'examen complémentaire des polymères irradiés dans le proche infrarouge.
- c) la possibilité d'utiliser l'activité de la peroxydase de choux de Bruxelles comme moyen de détection de l'irradiation de ce légume.

Résultats du projet n° 1

Directeurs des recherches et collaborateurs scientifiques : Prof. Ir. E. Maes, Prof. Ir. A.R. Deschreider, J.-M. Vigneron, Dr. Sc.

Titre du projet : La détection de l'irradiation des denrées alimentaires.

a) Infrarouge différentiel des plastiques irradiés.

Cette étude a permis de classer les polymères en trois groupes selon leur réaction aux rayons gamma.

Dans un premier groupe se trouvent le polystyrène, le propylène et le polyéthylène.

Le polyéthylène présente un nombre de bandes d'absorption ayant une absorbance supérieure à 10 %, qui croît en fonction de la dose d'irradiation. Cela se vérifie pour chaque épaisseur des feuilles de polymère.

Pour le propylène et le polystyrène également le nombre de bandes croît avec la dose et un certain nombre d'entre elles sont caractéristiques.

Il ressort que le polyéthylène et le polystyrène sont plus sensibles au rayonnement que le polypropylène.

Le second groupe comprend le chlorure de polyvinyle et la polyamide. Ici, le nombre de bandes d'absorption différentielle n'est pas aussi important ni marqué que pour le premier groupe.

Le troisième groupe comprend le cellophane qui ne présente que peu de bandes différentielles, mais elles sont très prononcées, en particulier celle située à 6 microns.

b) L'infrarouge proche des plastiques irradiés.

L'irradiation du polyester se traduit par un effet hypsochrome et l'apparition d'une bande d'absorption supplémentaire aux doses de 0,1 et 1 Mrad et de deux bandes supplémentaires pour 5 Mrad.

Lorsque l'épaisseur du film passe de 13 à 26 microns, le nombre de bandes décroît fortement. Pour le polystyrène, la zone la plus intéressante se situe entre 2,21 et 2,36 microns; en plus d'un effet bathochrome, une nouvelle bande d'absorption apparaît déjà à 0,1 Mrad.

Quant au cellophane, l'action du rayonnement dépend du type. C'est ainsi que pour le type 340 X S il y a un effet bathochrome prononcé après l'irradiation et disparition d'une bande d'absorption. Alors que pour le type 335 MS il y a un effet hypsochrome s'accompagnant d'une bande supplémentaire dont l'absorbance est proportionnelle à la dose de rayonnement.

En résumé, le choix des paramètres instrumentaux a permis d'établir une distinction entre les polymères irradiés et non irradiés.

c) L'activité de la peroxydase du chou de Bruxelles.

L'activité de la peroxydase de Brassica Obraceca a été suivie par la mesure de l'absorbance des solutions à 420 nm en fonction du temps.

Si les échantillons irradiés ont fourni des courbes d'activité de la peroxydase différentes de celles des échantillons non irradiés, il s'avère cependant qu'elles ne peuvent servir à la détection de l'irradiation de ce légume.

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

Contrat N° 061-72-1

Commissariat à l'Energie Atomique, Paris, France

" Niveaux de pollution du milieu ambiant "

Chef du Groupe de recherche : C. LACOURLY

Dans son stade actuel, l'étude comprend quatre projets principaux :

- 1/ Etude des paramètres biologiques de l'homme européen
- 2/ Etude des paramètres de transfert de la contamination de l'environnement à partir de la pollution de l'atmosphère
- 3/ Etude des paramètres de transfert de la contamination à partir de la pollution des eaux
- 4/ Etude des paramètres de transfert de la contamination déposée dans les sols.

Projet N° 1 -

Etude des paramètres biologiques de l'homme européen -
M. KARHAUSEN, Mme GARNIER -

RESULTATS -

1 - Paramètres de dilution au cours du transit gastro-intestinal
(collaboration du Professeur Loeb de l'Université de Bruxelles) -

L'étude de la dilution du repas chez le nouveau-né, l'enfant et l'adolescent à différents niveaux du tractus gastro-intestinal est terminée. L'introduction des valeurs obtenues dans le modèle mathématique, précédemment mis au point, permettra d'obtenir le premier modèle dosimétrique du TGI chez l'enfant.

2 - Métabolisme thyroïdien (collaboration du Dr Ermans, Decoster et Thilly de l'Université de Bruxelles) -

Une première étude porte sur les variations géographiques de la captation thyroïdienne et de l'apport d'iode stable dans les six pays de la Communauté Européenne. Une carte a été établie qui montre les variations régionales de ces valeurs. On observe des variations relativement importantes qui atteignent un facteur de trois entre les différentes régions. Il reste quelques zones d'ombre plus difficiles à connaître parce que moins bien desservies médicalement. Elles seront étudiées au cours de l'année 1973.

L'étude qui porte sur l'hétérogénéité de la distribution de l'iode dans la thyroïde est en cours. Cette étude doit permettre de calculer la dose délivrée non plus à la glande thyroïde, mais aux structures tissulaires.

Enfin, l'étude cinétique précoce de l'iode destinée à compléter le modèle métabolique antérieurement élaboré est terminée.

3 - Métabolisme du strontium (collaboration du Professeur Lanzola de l'Université de Pavie) -

Les résultats de l'enquête sur le strontium 90 dans les os des enfants ont été présentés au Congrès International sur le métabolisme du strontium à Glasgow.

4 - Absorption intestinale du plomb -

Une discussion critique de la littérature portant sur l'absorption intestinale du plomb chez l'homme a été présentée au Congrès organisé par l'Environmental Protection Agency et la C.E.E. à Amsterdam.

5 - Composition minérale du corps humain (Laboratoire de Chimie Analytique C.E.A., Fontenay-aux-Roses) -

Le programme comporte l'étude de la teneur des différents tissus du corps humain en oligoéléments. L'étude pilote est terminée et les techniques sont à présent au point.

Projet N° 2 -

Etude des paramètres de transfert de la contamination de l'environnement à partir de la pollution de l'atmosphère -

M. ANGELETTI -

RESULTATS -

Les recherches entreprises avec la collaboration du Kernforschungsanlage à Jülich (Dr Vogt) ont été poursuivies.

Au cours de l'année 1972, on a effectué, d'une part, des mesures in situ de la vitesse de dépôt sur l'herbe de l'iode I27 et, d'autre part, des mesures en système fermé (boîte à gants) du rapport entre les vitesses de dépôt sur l'herbe de l'iode et du iodure de méthyle.

Les essais in situ ont été réalisés tant sur des prairies naturelles que sur du ray-grass et du trèfle cultivé.

Les nombreux résultats obtenus sont encore en cours d'élaboration et feront prochainement l'objet d'une publication.

Les valeurs trouvées pour les vitesses de dépôt sont comprises dans l'intervalle de 0,3 - 2,7 cm s⁻¹ et elles apparaissent dépendre de la vitesse et du profil du vent ainsi que de la densité de l'herbe, de l'espèce végétale et de son âge. Le traitement en cours des données devrait permettre d'évaluer l'influence de ces différents paramètres.

Les études en système fermé ont montré que les valeurs du rapport des vitesses de dépôt de l'iode vapeur et de l'iodure de méthyle sont d'environ 80, ce qui est en bon accord avec ce qui a été trouvé par les expérimentateurs américains.

Projet N° 3 -

Etude des paramètres des transferts à l'homme de la contamination à partir de la pollution des eaux -

M. BITTEL, Mme VAUBERT, Mme GARNIER -

1 - Transferts de la contamination aux végétaux irrigués -

En collaboration avec la Division de Biologie d'Euratom à Ispra, deux études ont été poursuivies. La première concerne la contamination, par le chrome 51 sous forme chromate, de prairies de type " marcites " irriguées par submersion. Les coupes successives ne prélèvent qu'une assez petite fraction du chrome 51 apporté, cette fraction étant d'autant plus faible que la teneur des eaux en chrome stable est moins élevée ; mais les arrières effets d'une contamination sur les coupes ultérieures irriguées avec de l'eau non contaminée sont importants.

La seconde étude concerne la contamination, par le zinc 65 et le césium 134, d'une prairie mixte (ray-grass et trèfle) irriguée par aspersion. Les deux plantes se contaminent identiquement, les rétentions de deux nucléides sont égales, dans les conditions expérimentales adoptées, mais le césium migre plus dans la plante que le zinc.

En collaboration avec le laboratoire de Radioécologie Continentale du Département de Protection du C.E.A., on a réalisé à Cadarache des études sur l'influence de la minéralisation de l'eau d'irrigation et de la forme physico-chimique du radiocobalt sur la contamination de légumes irrigués par aspersion. La contamination des parties comestibles (feuilles de laitue, gousses de haricots) est plus faible quand l'eau est fortement minéralisée et quand le cobalt est complexé.

2 - Transferts de la contamination aux organismes dulçaquicoles -

En collaboration avec la Division de Biologie d'Euratom à Ispra, on a poursuivi les études sur la contamination des poissons par le zinc 65 et entrepris des recherches relatives au chrome 51. On a constaté en particulier que le chrome III était plus " accumulé " que le chrome VI.

L'étude de la contamination d'invertébrés par divers produits d'activation (zinc, cobalt, chrome) a montré que les formes complexes étaient moins prélevées que les formes simples.

3 - Essais d'évaluation des risques de contamination radioactive interne de l'homme du fait de l'ingestion d'aliments d'origine marine -

En utilisant les données de la Radioécologie et des enquêtes alimentaires, on a comparé la méthode dite des facteurs de concentration et celle de l'activité spécifique. Appliquées à un cas concret, elles conduisent à des résultats du même ordre.

4 - Transfert de la contamination au cours des processus de production et de transformation des produits alimentaires -

En collaboration avec le Centre National de Recherche Zootechnique et avec le laboratoire de Biologie Végétale du Département de Recherche Fondamentale du C.E.A., on a étudié le comportement de divers métaux lourds (Hg, Pb, Cu, Zn, Cr, Co, Fe) des aliments du bétail (vache) au lait, puis à divers produits dérivés du lait. On constate une concentration de ces éléments dans les fromages et une dilution relative dans les lactosérums correspondants.

5 - Transferts des pollutions associées aux pollutions radioactives dans des chaînes trophodynamiques marines -

En collaboration avec le CERBOM à Nice, on a étudié le transfert de divers métaux lourds (Pb, Hg, Cr, Cu, Zn) dans une chaîne du type benthique à mollusques. On constate une forte concentration relative dans les deux échelons marins étudiés (plancton, moules). Dans les conditions expérimentales adoptées, l'incidence de la forme chimique des polluants est faible.

Projet N° 4

Etude des paramètres de transfert de la contamination déposée dans les sols et les limons -

M. MAGNAVAL -

RESULTATS -

1 - Etude du comportement du césium 137 dans les sols -

L'évaluation du transfert sol-plante à partir d'expériences en conditions contrôlées est comparée aux résultats déduits d'observations in situ. Les valeurs observées sont proches dans les deux cas, pour des teneurs en césium échangeable inférieures à 1%, au-delà le coefficient de transfert observé in situ est toujours inférieur à celui déterminé à partir d'expériences en pots.

Les caractéristiques physico-chimiques du sol : capacité d'échange, teneurs en argile et en matières organiques, influencent l'absorption du césium 137 dans le cas du blé, par contre, la contamination indirecte de l'herbe est beaucoup plus dépendante de la teneur en eau du sol et des facteurs saisonniers.

2 - Fixation du mercure 203 sur les sédiments (collaboration aux travaux du laboratoire de Biologie Végétale du CEN-Grenoble) -

La cinétique de fixation du chlorure et du méthyle de mercure par les sédiments de rivière est de la forme

$$C = C_0 \left(1 + \frac{t}{a} \right)^n$$

où C_0 est la concentration initiale et C la concentration à l'instant t du polluant ".a et n " sont des paramètres dépendant du modèle expérimental.

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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

Results:

The results of 6 of the projects are organized according to the different levels of the food chain, starting from the physical and chemical environmental factors of the environment. The last project represents a joint effort to synthesize the results obtained by all projects in order to build short-term and long-term models of the behaviour of radioisotopes in the marine environment. The long range program of each project contains at least two parts, one to be carried out under laboratory conditions and one under field conditions in order to guarantee that the experiments are carried out under conditions comparable to those in nature.

As in previous years the isotope work has been centered on zinc and phosphorus. Zinc is representative for a metal with different physicochemical states, both labil and inert, while phosphorus is an example for a biological important element with a rapid turnover .

Since many publications on up-take and loss experiments do not supply enough informations on such basic and important biological data such as growth, culture conditions etc. , great emphasis has been placed on the importance of these parameters in the projects.

Project No. 1

Title: Physical environmental factors of marine contamination and
Special Developments

Name of scientists: M. Bernhard, A. Benedetti, F. Moller and
M. Soldi (all part-time)

Results:

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

The dynamics of particles, inorganic and organic, play an important role as sorption sites in the distribution of radioisotopes.

Phytoplankton with fluorescent chlorophyll represents probably the most important way of entrance of elements into the food chain. In order to select a counter for the instrument system the results obtained with three commercial available instruments (Celloscope, "Cell counter", (Air supply), Cytofluorograph) were compared with the manual counts using the Utermoehl technique (in collaboration with project 3). Of the three instruments only the Cytofluorograph is already capable to distinguish between fluorescent and not fluorescent particles the other two have still to be adapted. Comparing the results testing mono-and-poly-algal solution showed that the "cell counter" net values were about 7 % higher than the manual counts with a coefficient of variance of less than 2 % (2000 events). The counts obtained with the Celloscope which had been interfaced by us to a 100-channel-analyser to obtain a frequency/size distribution, were 5 % higher than the manual counts when clean cultures were counted. The Cytofluorograph demonstrated that algae with marked size differences could be distinguished by their size and their chlorophyll content, but the number of events did not correspond to the values obtained by manual counts.

Studying the flowsystem of the Cytofluorograph which has the most promising setup for a particle counter system indicated that the sample flow can be focused into the light detection beam if the flow of the sample and that of the supporting flow media are adjusted appropriately.

A new sequential counting strategy has been developed which will reduce the counting time necessary for the classic Utermoehl method used in phytoplankton counting considerably (in collaboration with project 3).

2) Simulation and model building

As contribution to project 7 several computer programmes have been newly written or adapted.

- a) Evaluation of transport rates between two and three compartment closed system not in steady state from experimental data.
- b) Simulation of non-steady-state compartment system up to 4 compartments.
- c) The 'Basic' of the computer laben 70 has been extended with subroutines for punch-tape-input, punch-tape-output and for an output in XY graphic form with one variable versus three.
- d) Adaptation to the IBM 360/65 (Pisa) of the 'Matexp' program for the numerical solution for ordinary differential equations with an extension to non-linear equations.
- e) Comparison of various IBM-Programmes for the numerical solution of non-linear differential equations on the IBM 360 (Pisa).

3) Instrumentation and computer programs needed by other groups

Computer programmes for the evaluation of sequentially obtained counts of phytoplankton algae according to a new counting strategy applied to the Utermoehl method (see also project 3).

Interfacing of a IBM correspondence teletypewriter to Laben 70 to be used for the editing of publications (administration).

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 reactions of equilibrium of the physicochemical states of zinc in seawater mainly by anodic stripping voltametry

Zinc in seawater was found to be present (see Annual Reports, 1968 to 1971) in three main physicochemical states: complexed, particulated and ionic. The investigation has now been focussed on the equilibrium reactions between these forms. The natural equilibrium can be disturbed by addition or subtraction of zinc in one or more physicochemical states. After subtraction of the ionic fraction by controlled electrolysis, a pronounced influence of the pH has been observed. At pH 6 the equilibrium is reestablished by release of zinc from the complexed fraction to the ionic one. At pH 8, even after several days, the redistribution does not occur. This is in agreement and could explain the fact that the ionic radioactive zinc added to seawater at pH 8, does not enter in equilibrium with the complexed fraction (A. Piro et al, 1972). In particular, when radioactive ionic zinc is added to seawater at pH 8 a part (~ 50 %) of this remains in solution in the ionic form and the remaining part is transformed into the particulate form. Consequently for marine organisms which, for example, selectively accumulate zinc in the ionic form the available zinc will have a higher specific activity than that estimated on the basis of the total concentration of zinc in seawater.

2) Investigation of the influence of storage conditions in the iron distribution in seawater

During the development of the automated method for iron determination in seawater it has been found that different conditions of storage can produce wrong results on the determination of the iron content of a seawater sample. The adsorption of iron on the surface of the storage bottles

has been investigated as function of the following parameters:

- kind of container;
- temperature of storage;
- acidification of the sample to pH 2.

Polyethylene and Pyrex glass bottles have been used in the experiment. Iron is quickly adsorbed from normal seawater even at 0°C, this process being slower using Pyrex bottles than Polyethylene bottles. Changing the storage temperature from 0°C to 20°C the adsorption on the plastic bottles increases gradually and after 40 days only 30 % of the iron originally present is still in solution. On the contrary the adsorption on the Pyrex bottles is practically not influenced by the change of temperature, having reached the adsorption equilibrium during the storage at 0°C. If seawater is acidified to pH 2 and stored at 0°C there is no loss of iron by adsorption, but at 20°C the iron content increases very quickly especially in the seawater stored in plastic bottles. Experiments to confirm the possibility of a release of iron from the containers under acid conditions have been planned for the next future.

3) Automated colorimetric method for direct zinc determination in seawater

The anodic stripping voltametry has been successfully used to measure zinc content of seawater, as well as its physicochemical states. Unfortunately this method is time consuming requiring 1 to 4 hours for each determination. Consequently efforts were made to develop an automatic colorimetric method to determine the concentration of total, and ionic plus particulate zinc in seawater. The sample should be completely mineralized under high temperature and pressure (this step is necessary only for the "total" zinc determination), and allowed to react under controlled conditions with a dithizone hydroalcoholic solution.

From preliminary results the automated method shows a sensitivity of 10^{-8} $\mu\text{gat Zn/l}$ and offers a capacity of carrying out 30 to 40 analyses per day.

Project No. 3

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

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

The scope of the project is to collect information on the most important parameters which can influence uptake, loss and transfer along the food chain of isotopes (stable and radioactive), on the stable element content in order to predict the possible level of contamination and on the population dynamics of primary producers.

1) Uptake and loss of stable and radioactive zinc

Results on uptake of zinc in a system which simulates a not growing algae culture (chelex 100) showed different distribution patterns between stable and radioactive zinc. This phenomenon due to different physicochemical forms must be taken into consideration in evaluating data on uptake of radioactive isotopes under natural and laboratory conditions.

Experiments carried out to establish if metabolic products of algae (*Phaeodactylum tricornutum*) can change the physicochemical states of zinc showed that no change occurred. Uptake of zinc in algae (*Platymonas suecica*) is related to the zinc present in the medium. The fluxes, or transport rates, of zinc have been evaluated utilizing a closed two compartments model not in steady state.

2) Uptake of phosphorus

Light energy supplied influence significantly the population size and phosphorus accumulated by *Phaeodactylum tricornutum*.

The uptake of phosphorus from different sources of inorganic and organic phosphorus showed that algae can take up phosphorus both from inorganic and organic forms and when the two forms are present the algae take up preferentially the inorganic phosphorus. A two compartment closed system had been adapted to evaluate the transport rates in these experiments (Fig. 3.1 and 3.2).

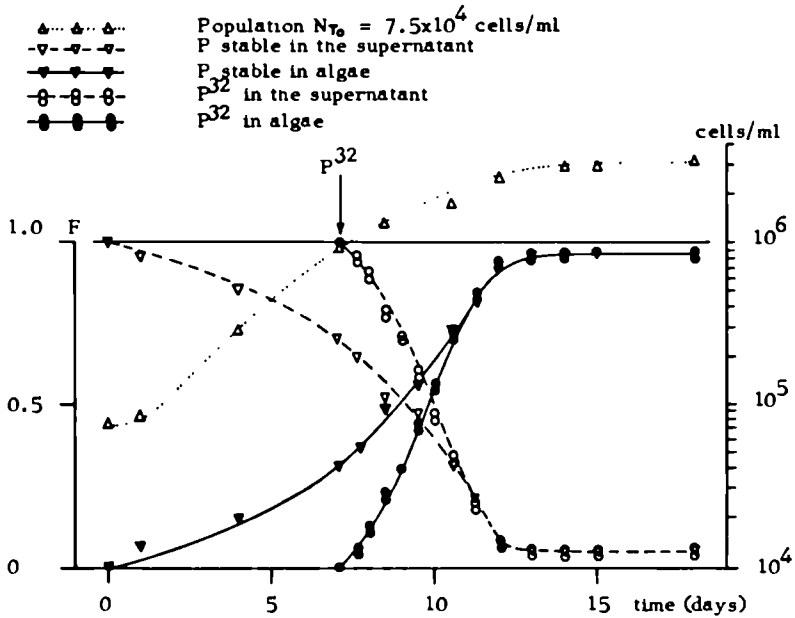


Fig. 3.1 - Uptake of stable and radioactive (P^{32}) phosphorous by *Phaeodactylum tricornutum*. Phosphorous is expressed as fraction of the total amount before inoculation (1.0). P^{32} was added at the 7th day from the beginning.

Preparation: $\left\{ \left[\left(RSW + P_{RSW} \right) \right]_{7d} + \text{algae} \right\}_{7d} + \left(P^{32} + P \text{ carrier} \right)$

$$\frac{\text{Total } P^{32}}{\text{Total stable P}} = \frac{5.45 \times 10^{-5} \mu\text{gat } P_T^{32}/l}{35 \mu\text{gat } P_T/l} = 1.6 \times 10^{-6}$$

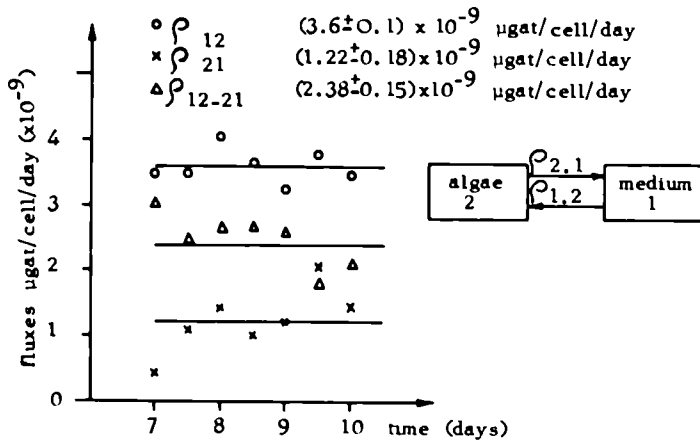


Fig. 3.2 - P uptake by *Phaeodactylum tricornutum*. Fluxes evaluation between days 7 and 10 (see fig. 3.1)

3) The chemical composition of phytoplankton

Natural and artificial populations have been prepared for radiactivation analysis. Particular attention will be given to those elements which possess analogous radioactive isotopes potentially dangerous.

4) Studies on phytoplankton populations

Growth characteristics (generation time and growth coefficient) have been evaluated in situ for natural populations and compared with populations composed from algae strains of our culture collection. The results show that the generation time and growth coefficient obtained in situ are practically the same as those under laboratory conditions. The knowledge of these parameters is of great importance in the estimation of the potential production in natural populations and hence in the potential accumulation of radionuclides of a given biomass.

As appears from nutrition studies with organisms in higher food chains the prediction of radiocontamination must be made at specie level.

Hence the right identification of an individual must have the same precision as the enumeration of the populations.

Therefore, a key which permits the right identification of a specie by a non-taxonomist has been so far prepared for Diatomeae and Peridineae found in the Mediterranean Sea.

A new time saving sequential approach for the microscopic counting of natural populations has been developed (in collaboration with project No. 1).

Project No. 4

Title: The role of the first heterotrophic levels (zooplankton) in the accumulation and transfer of radionuclides relevant to marine radiocontamination

Name of scientist: A. Nassogne

Results:

1) Accumulation and loss of stable and radioactive Zn by Euterpina a.

Stable and ^{65}Zn uptake through feeding was found nearly 10 times higher than direct uptake from water. By transferring Euterpina from laboratory mass culture (about 5.5 ug stable Zn in water and algae) into normal seawater (5.4 ug stable Zn/l) total Zn content of copepods decreases to reach an equilibrium after 4 days. Nearly 40 % of the initial Zn content was excreted while uptake represented only 0.8 %. Only particulate and ionic Zn were accumulated mainly through adsorption on Euterpina external surfaces. Increasing stable concentration in seawater, stable Zn uptake increased proportionally, reaching in 90 ug stable Zn/l solution about 8 % of the stable Zn initial content in copepod. Loss of stable Zn, however, is only slightly reduced so that the stable Zn concentration in Euterpina at equilibrium is only little increased by increasing stable Zn in solution and may be considered as regulated. In solution with algae, Euterpina reached at equilibrium a stable Zn or ^{65}Zn content proportional to the corresponding concentrations in algae. Thus no regulation was observed, even after a long period of loss in seawater. Zn accumulated from food is probably bounded into compartments characterized by a very slow Zn loss rate. Summarizing these results, showed that ^{65}Zn level in copepods could be predicted knowing stable Zn concentration, ^{65}Zn specific activity and physicochemical state of both ^{65}Zn and stable Zn in the medium (seawater and algae). A mathematical model will be developed in this direction.

2) Accumulation and loss of radioactive and stable P by Euterpina

(In collaboration with project 6)

Direct accumulation of ^{32}P from seawater is mainly due to bacterial flora associated with Euterpina a. Influence of bacteria however is neglectible when ^{32}P is accumulated through food. Uptake from algae is about 10 times higher than direct uptake from water, both reaching a plateau within 2 days. 50 % of ^{32}P accumulated from food or from water is lost in normal seawater with or without algae after few hours. In order to rely, in the future, data on ^{32}P to stable P content in animal and medium, a technique was developed for P determination in algae, seawater and copepods. Stable P content in Euterpina is not directly related to P content in algae or seawater.

These preliminary results allow to set up optimal experimental conditions for future experiments on phosphorus metabolism in copepods.

3) Microdistribution of radionuclides in Copepods by autoradiography

(In collaboration with Dr Debertoldi, Pisa University)

Techniques for including Euterpina a. in synthetic resins were successfully developed. Anatomical description using normal and electronic microscopy and influence of radionuclides concentration or exposure time on the quality of autoradiography are in course. Autoradiography will allow to localise "de visu" the accumulation pools of ^{32}P and ^{65}Zn in copepods according to different accumulation and loss ways.

4) Microdistribution of natural zooplankton

The continuous zooplankton sampler developed previously was further modified. Microdistribution of the most important species of copepods was followed in a series of 50 samples corresponding to successive sampling distances of about 80 m. Distribution was heterogeneous in the range of 0.9 to 4.5 organisms/l. Shorter distances have to be adopted in view to point out eventual patchiness in zooplankters distribution.

Project N. 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. Schulte

Results:

- 1) Culture experiments with mussels (Mytilus edulis) and shrimps (Leander squilla)

Both species were tried to culture under laboratory conditions as a steady source for further experiments on the transport of radioactive nuclides in the food chain. Conditioning for out-of-season spawning failed only in shrimps (extirpation of eyestalks) since oviposition did not include fertilization of the eggs. In mussels stimulation by temperature shock gained in successful spawning of both sexes within three hours. Larvae of mussels and shrimps were successfully hatched and cultured under different environmental conditions (temperature, food amount, population density). Best survival and growth rates were found for mussels larvae at 20°C and for shrimps larvae at 20°C and 25°C respectively. Populations-densities from 10-30 shrimp larvae per 1000 ml were the most favourable ones. A good continuous culture of shrimps is now established.

- 2) Life cycle and reproduction time

The breeding period (1972) or the reproduction time of Leander squilla in the sea lasted from the first days of May (female with external eggs) to the end of September (150 days). Females caught in the beginning of May and kept in the laboratory spawned during that period twice and again had eggs after 2-3 months interruption. First spawning of Leander squilla larvae, which were hatched to metamorphosis under laboratory conditions, occurred at an age of about 210 days, so that one life cycle from egg to egg lasted about 240 days under

laboratory conditions (20°C), while under natural conditions first spawning will take place not before April or May of the next year. The natural life cycle lasts according to that about one year.

3) Food requirements of mussels (Mytilus edulis)

In three series of experiments using single mussels, small groups of six mussels and big groups of 50 mussels (all together about 450 specimens) the filtering behaviour of Mytilus spec. was studied in order to determine the filtering capacity ("feeding rate"; "filtered volume") expressed in ml filtered per animal per hour. The influence of seven ascending pure algae concentrations (Platymonas suecica) reaching from 5×10^2 to 1.5×10^5 algae cells/ml on the "filtered volume" was tested by following up the decrease of the initial algal concentrations during time applying the "Utermoehl method" for algae counting. The filtering capacity (ml/h) was calculated with the Gauld formula:

$$F = v \cdot \frac{\log \text{conc } t_0 - \log \text{conc } t_1}{\log e \times t} \cdot 60$$

V = volume (ml of algae solution used; conc. t_0 = initial concentration; conc. t_1 = concentration at time t_1 ; t = time (min) between $t_0 - t_1$.

The results of all three series generally show a decreasing filtering activity with increasing algae concentrations. Solutions ranging from 5×10^2 to 10^3 algae cells/ml as well as from 10^5 to 1.5×10^5 cells/ml significantly depressed the filtering activities of mussels at a high rate, while those concentrations between 10^3 and 10^5 algae cell/ml only had a low effect on the filter capacity of Mytilus, so that the decrease of the filtered volume was very little considering the high change in algal concentrations. . .

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

1) Investigation of the role played by bacteria in the passing of radioactivity to higher trophic levels along the marine food chain

1a) Transfer of ^{32}P from bacteria to copepods (collaboration with project

No. 4)

The copepod Euterpina acutifrons made "sterile" by preincubation in seawater + penicillin 0.1 % and added with various amounts of labelled bacteria (strain 21-6-9, a rod) takes up as much activity as the blank. "Not sterile" copepods without added bacteria take up an activity more than 20 times higher than the blank.

The same results have been obtained by using a staphilococcus (strain 25-5-19) instead of a rod as labelled strain. Nauplii behave in the same manner as adult Euterpina under the same conditions.

If labelled bacteria are added to "sterile" copepods in the presence of algae (β_2), used as food by Euterpina, a quantity of ^{32}P significantly higher than the blank was taken up.

This indicates that the bacteria present in the environment are unimportant as food and, therefore, as transfer agent of radioactivity as far as Euterpina acutifrons is concerned, whereas bacterial flora associated with the copepod is very important.

Bacteria present in the environment can be important in presence of algae ingested by the copepods, probably due to their adhesion to the algal body.

1b) Transfer of ^{32}P from bacteria to mussels

It was observed that mussels pretreated with antibiotics take up less activity than untreated mussels probably due to the epizootic microflora living on the shell. Therefore, pretreated mussels have been used in the further experiments.

The results indicate that mussels accumulate radioactivity mainly in pseudofaeces and to a lesser degree also in soft tissues.

This means that an ingestion of bacteria occurs and a moderate assimilation of radiophosphorus from bacteria can take place.

2) Role of bacteria in the uptake of radionuclides

2a) Dynamic of ^{32}P uptake in laboratory conditions

Experiments have been set up to investigate the dynamic of uptake of radiophosphorus by bacterial strain 21-6-9.

By incubating in active sterile seawater a heavy bacterial suspension previously grown in medium at high stable P content, it was observed that the bacteria lose P exponentially and the ^{32}P reaches immediately an equilibrium between bacterial cells and seawater.

By incubating in active sterile seawater few bacterial cells, a close correlation was observed between uptake of stable and radioactive P by bacteria and the disappearance of stable and active P from the seawater, as the number of bacterial cells was increasing.

2b) Effect of hydrostatic pressure on ^{32}P uptake

By using a pressure chamber, the effect of moderate pressure (40 Atm) was studied on the radioactive P uptake by a surface strain (λ) and a deep-sea strain (4Z-3-4).

The uptake in the surface strain was markedly inhibited in comparison to the uptake at environmental pressure, but it was unaffected in the case of the deep-sea strain.

This indicates that hydrostatic pressure is an important ecological factor involved in the decreasing of microbial activity with the depth.

Strain 4Z-3-4 will be used for in situ experiments to investigate the role of ecological factors other than pressure.

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 compartments systems

In order to prepare for the treatment of the experimental data obtained in the other projects, computer programs have been developed or adapted and tested which can be used for the interpretation of closed 2, 3 and 4 compartment models not in steady state (see project 1). If simulations based on experimental data obtained under certain conditions are run at different growth rates and different initial population size the radiocontamination due to growth accumulation can be compared with the contamination due to exchange. As one could expect in fast growing organisms growth accumulation is by far more important than exchange accumulation. Therefore for the estimation of transport rates per example it was possible by simulating various parameters such as initial population size, concentration of stable carrier, sampling frequency etc. to improve on the design of successive experiments.

Simulation of an entire tracer experiment showed in fact that the precision of the estimation of the transport rates can be greatly improved if the optimal sampling schedule is first determined by a simulation. It also demonstrated quite clearly that more precise data are needed for an estimation of transport rates than usually supplied by the experimenters.

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

The survey had to be postponed due to lack of external means.

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Collaborations 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 (Institut Ruder Boskovic) Rovinj, Yugoslavia.

The 14th Contact Group Meeting on Marine Radioactivity was held in Rovinj, February 1972. At this meeting participated besides staff members of the above mentioned laboratories, scientists from Fisheries Radiobiological Laboratory, Lowestoft, England and the Radiobiological Laboratory of the Biologische Anstalt Helgoland, Hamburg, Germany.

Drs. Bernhard and Piro participated in the Symposium on the "Interaction of Radioactive Contaminants with the Constituents of the Marine Environment", Seattle, Washington, July 1972. On this occasion they visited the following institutions: Woods Hole Oceanographic Institution; Department of Oceanography, University of Seattle; Battelle-Pacific Northwest, Richland, Washington; School of Oceanography, Oregon State University; Scripps Institution of Oceanography, La Jolla; Radiobiological Laboratory, Beaufort, N.C.

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

Nummer van het contract: 094/72/1 BIAN

Hoofd van de researchteams: 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, physiological).
-

Algemene omschrijving van de uitgevoerde werkzaamheden:

Main topics of the 1972-research by the soils and plantgroup of the Institute were:

- Sampling and analysis for control of the simulation model concerning the behaviour of ^{90}Sr and ^{137}Cs in soils of Western Europe.
- Tracing of soil moisture movement with ^{36}Cl , ^{60}Co and Tritium and considering the influence of water movement on the translocation of tritium and tritiated compounds in soils.
- Transport and behaviour of ^{51}Cr , stable chromium, mercury and mercury compounds in soils.
- Kinetics of the uptake of ^{51}Cr and stable chromium, on the one hand, by intact plants and, on the other hand, by chloroplasts.

The work on chromium, mercury in soils and plants is part of a research programme on heavy metals in the food chain in collaboration with the Biology Division at Ispra.

Other research topics were:

- Improvement and testing of a new molecular theory on radiation effects in biological material. The theory has been successfully used to analyse the variation of radiation sensitivity in the cell cycle and to explain observed chromosome aberrations.
- Development of new or improved dosimeters and their application in biological research.
- Although the research on the irradiation dose-fractionation effect in *Saintpaulia* is part of the Applications programme

it has to be mentioned here that research on changes in the metabolic activity and on the induction of a "protective" agent is in progress.

Other information from the Applications programme, using *Lemma minor* populations grown on either ^{14}N or ^{15}N and either ^{10}B or ^{11}B solutions and chronically irradiated with thermal neutrons, show more sensitivity to boron than to N-substitution. Damages, due to the N,p (^{14}N) reaction, however, more severely impair the subsequent growth than damages due to the N, α (^{10}B) reaction.

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Management Committee

G. Wansink	chairman
P. Buringh	vice-chairman
R.K. Appleyard	secretary
R. Craps	
A.C. Schuffelen	

At the end of 1972 Prof.Dr.Ir. P. Buringh retired from the Board and the Management Committee. His successor is Ir. J.B. Ritzema van Ikema (President of the Board of the Agricultural University).

International Scientific Advisory Council

A.C. Schuffelen	chairman
F. D'Amato	vice-chairman
H. Laudelout	
H. Laven	
H.F. Linskens	
H. Marschner	
W. Pilnik	
G.F. Wilmink	

In the course of 1972, Prof.Dr. F. D'Amato and Prof.Dr. W. Pilnik, respectively vice-chairman and member, retired from the council.

Changes in the Scientific Staff

- Ir. J. Beek, Dr.Ir. R.B. Contant, Dr. R.M. Ecochard and Drs.Mrs. K.J.A. Wijnands-Stäb, have left the Institute, mainly to accept research or teaching duties elsewhere in the world.
- New members of the scientific staff are: Dr.Ir. J. Sinnaeve and Drs. G. Desmet, both from Belgium.

- Temporary members (post-graduate fellows) responsible for particular aspects of the programme: Ir. Miss H.M.G. Groot, Miss M. Matteoli, Ir. C. Petit, Ir. J.P. Rolland and Ir. M. van de Steene.
- Several guest-workers, mainly from developing countries have spent 6 to 12 months at the Institute.

The programme for 1972 has once more been carried out in close cooperation with other scientific institutes wherever possible. Examples of this scientific collaboration are:

- on heavy elements in the food chain with the Biology Division in Ispra and within the European Society of Nuclear Methods in Agriculture (ESNA);
- on mutation breeding, incompatibility, protein content and disease resistance in higher plants in the Mutation Breeding Contact Group;
- on pollution problems with Institutes in the Netherlands, Germany and Belgium and with the ESNA working group;
- on radiation effects within the European Dosimetry Working Group;
- on standardization of absorbed dose and dose distribution measurements within the European Late Effects Project Group (EULEP);
- on incompatibility in higher plants with several Institutes and Organizations in Italy and the Netherlands;
- on improvement of the "wholesomeness-testing" technique in collaboration with Institutes in Denmark and the Netherlands;
- cooperation to projects concerning the testing of irradiated food, set up by the Organization for Economic Cooperation and Development (OECD) and the International Atomic Energy Agency (IAEA);
- on applications of the food irradiation technique with different Institutes in the Netherlands;
- research on genetic control of insect pests, coordinated, nationally in Section VII of the TNO Working Group "Integrated Control of Insect Pests" and, internationally in the joint European Working Group of the "Organisation Internationale de la lutte Biologique" (OILB) and of ESNA.

Other interesting contacts are those within the German-Dutch cooperation, mainly on food-irradiation, and with groups in the Agricultural Faculty of the Catholic University at Louvain.

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 (mainly derived from computer simulation models) concerning ^{90}Sr and ^{137}Cs behaviour in soils of Western Europe

Beschrijving van de resultaten:

As indicated by the title the main object of this project is control by field sampling of the calculated information on ^{90}Sr and ^{137}Cs behaviour, which was derived from the simulation model. Ten sampling sites, 2 in Germany, 2 in France, 2 in Italy and 4 in the Netherlands, covering the most important European types of soils, have been maintained in this project. To work off arrears in the analysis of soil and plant samples (mainly 1971) it had been decided to interrupt the sampling for one year in 1972. Due to an accident with the special drying oven, half of the 1971 samples, which were in the final stage of preparation for analysis, have been lost. The analysis of the remaining 1971 samples is now in progress. Owing to the high costs of the sampling and the analysis and, notwithstanding this 1971 drawback, the decision concerning the 1972 sampling has been maintained.

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project No. 2.

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra, F. van Dorp.

Titel van het project: Transport of soil moisture and its effect on the regime of tritiated water and other tritium compounds.

Beschrijving van de resultaten:

Tracing soil moisture migration with ^{36}Cl , ^{60}Co and Tritium.

Dispersion effects of solutes in the liquid phase of soils can be studied by tracing the migration of water. Three radioactive isotopes were selected for this purpose: ^3H to label H_2O , ^{36}Cl to label Cl^- ions and ^{60}Co to label $\text{Co}(\text{CN})_6^{3-}$.

The chloride ion was selected because, in several studies on the water-balance of soils, the migration of chloride is taken as representative for the migration of water. With ^{36}Cl and ^{60}Co inorganic anions are labeled, but it is generally assumed that these may be used to trace the migration of water in systems like soils, that do not adsorb these anions. Tritium has been chosen because it has the advantage that it can be used to label the water-molecules themselves. The disadvantage of tritium is that isotopic exchange with organic substances in the soil may occur. Such an exchange would cause a delay in the tritium migration compared to the water migration.

The mentioned dispersion studies are being performed in undisturbed soil columns with a diameter of 12 cm. It is a great advantage for such studies if the water migration in the columns can be followed without disturbing the soil. ^{60}Co emits a γ -radiation of 1.33 MeV and thus permits such a measurement by scanning the $\text{Co}(\text{CN})_6^{3-}$ ions from the outside. The emission of ^3H and ^{36}Cl is far too weak for such purpose.

The experimental work has partly been carried out by K. Harmsen, of the Laboratory of Soils and Fertilizers of the Agr. Univ. (Dept. of Prof. G.H. Bolt).

Breakthrough curves. Two columns filled with a clay soil (a river foreland clay soil or fluventic eutrochrept) and two columns filled with a sandy soil (old arable land or plaggept) were treated with "rain" (flux 1.14 cm d^{-1}) long enough to establish steady state conditions. This flux is much higher than in nature; to avoid the danger of structure degradation associated with complete removal of salts from the soil, the percolating solution was given approximately the same ionic composition as the original soil solution (0.003 N CaCl_2 , 0.001 N KCl , 0.001 N NaCl).

Next, mixtures of the salts of $^{36}\text{Cl}^-$ and $^{60}\text{Co}(\text{CN})_6^{3-}$ (same cation ratio as above) and $^3\text{H}_2\text{O}$ were supplied during 32 days to the sandy soil and during 40 days to the clay soil. Thereupon leaching was continued with the original solution. The effluent was analysed two times per day for

^{36}Cl , ^{60}Co and ^3H by liquid scintillation counting.

Results

The calculated curves were obtained with a simulation model taking into account both diffusion and dispersion and applicable to heterogeneous one dimensional systems (cf ref. 1). In this calculation diffusion and dispersion are combined into an apparent diffusion coefficient according to $D_A = \theta \cdot \gamma \cdot D + \theta \cdot d \cdot V$, in which θ equals the volumetric water content, γ the tortuosity of the soil and D the diffusion coefficient in pure water. If D is given in $\text{cm}^2 \text{sec}^{-1}$ it is convenient to express the dispersion "distance", d , in cm and the linear velocity of the solution in the column, V , in cm sec^{-1} ($V = \text{waterflux}/\theta$).

For the present experiment the water fraction in the clay soil varied from 0.61 at the top to 0.45 at the bottom; the total water content of the column was 5713 ml. The water fraction of the sandy soil varied from 0.37 at the top, to 0.15 in the center of the column and to 0.30 at the bottom; the total water content was 2586 ml. The calculated curves corresponded with a mean dispersion distance of 4 cm for the clay and 5 cm for the sand. The sensitivity of the spreading for variation of the dispersion distance is shown in fig. 1: both, the calculated curves, for a dispersion distance of 2 cm and 6 cm are included.

For both, clay and sand, there is sufficient agreement between experimental and calculated values to conclude that adsorption of Tritium is negligible. The fit between calculations and experiments is less good for Cl^- ions, but taking into account that undisturbed soils were investigated the results are accurate enough to conclude that the use of chloride ions as a tracer for water would for the present soils lead to errors of 11 percent for the sandy soil and 37 percent for the clay due to anion exclusion or desorption. The $^{60}\text{Co}(\text{CN})_6^{3-}$ ions show approximately the same deviation. Due to radiochemical impurities (or decomposition during the experiment?) part of the ^{60}Co appeared to be adsorbed as $^{60}\text{Co}^{+++}$ ions, as was verified in the column. The maximum value of the ^{60}Co breakthrough curve was therefore lower than that of the ^{36}Cl breakthrough curve

In as far as the slight trailing behind of $^{60}\text{Co}(\text{CN})_6^{3-}$ in comparison to $^{36}\text{Cl}^-$ - as noticed especially in the clay column - is significant, this might imply that some decomposition of $^{60}\text{Co}(\text{CN})_6^{3-}$ took place during the experiment.

From the above the conclusion appears warranted that tritiated water is far superior to anionic tracers for following the migration of water in soil. As expected the latter exhibit clearly the effect of anion exclusion by negatively charged soil colloids, leading to premature breakthrough. As was shown, this effect may be quite significant in clay soils. The presence of Co^{+++} impurities in solutions of $\text{Co}(\text{CN})_6^{3-}$ may cause further difficulties in the interpretation of the results. Nevertheless $\text{Co}(\text{CN})_6^{3-}$ being "scannable", remains a useful tracer, if corrections for the anion exclusion and decomposition are made.

Adsorption of tritium or isotopic exchange of tritium with H-atoms from soil constituents seems to be insignificant.

Submitted for publication in Nature: M.J. Frissel, P. Poelstra, K. Harmsen, G.H. Bolt, Tracing soil moisture migration with ^{36}Cl , ^{60}Co and tritium.

Annual report of the Association EURATOM-ITAL.

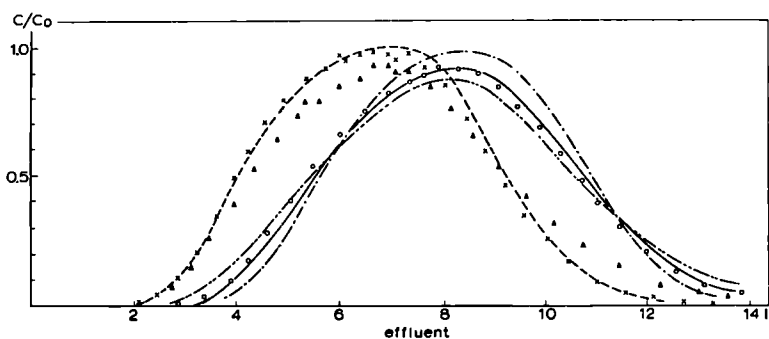


Fig. 1 Breakthrough curves for a clay soil.

Experimental values: 0 : ^3HHO , + : ^{36}Cl , Δ : $^{60}\text{Co}(\text{CN})_6^{3-}$.

Calculations: ————— : ^3HHO , dispersion 'distance' 4 cm,
no adsorption.

— · — : ^3HHO , dispersion 'distance' 2 cm,
no adsorption.

— " — : ^3HHO , dispersion 'distance' 6 cm,
no adsorption.

— - - - : $^{36}\text{Cl}^-$, dispersion 'distance' 4 cm,
desorption 37 percent.

Resultaten van het project No. 3.

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel and P. Poelstra.

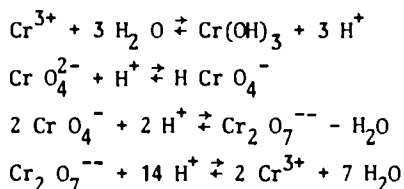
Titel van het project: Transport of chromium and other heavy metals (mercury excluded) in the soil.

Beschrijving van de resultaten:

Transport and accumulation of chromium in soil.

The chromium regime in soils depends mainly on the chemical form. From our leaching experiments it appeared that, from the Cr^{+++} ions, more than 90% had been adsorbed by the soil, irrespective of the soil type. The CrO_4^{--} adsorption varied between 0.5 and 95%, depending on the soil type.

For soils the following equilibria are important:



In HCrO_4^- chromium can be considered as Cr^{6+} and thought of as dissociated in H_2O and $\text{Cr}_2\text{O}_7^{--}$

With the equilibria constants of these equations a Redox potential - pH diagram can be set up which shows the relation between chemical form, Redox potential and pH.

Boundaries are formed by concentrations at a 10^{-3} molar level (50 ppm) and 10^{-4} molar level (5 ppm) (see fig. 1).

Reiniger has found redox potentials varying between 20 to 40 mV for aerobic soils and values of -90 to 180 mV for anaerobic soils. From the diagram and those measurements it can be concluded that a major part of the Cr-ions will be precipitated in soils as $\text{Cr}(\text{OH})_3$. Only at low pH values, very high redox potentials and very low Cr concentrations the major part of the Cr remains in solution. The diagram shows that in peat soil part of Cr may be present as anions.

The diagram gives no information on the velocity with which the compounds are oxidized, reduced or precipitated. So it is possible that a solution of K_2CrO_4 , which is expected to precipitate, can still be leached out or taken up by plants if the precipitation rate is slow.

Leaching experiments were carried out both with Cr^{+++} and CrO_4^{--} .
As references purified sand and Dowex-50 were used.

Characteristics of the soils:

1. Valburg, area frequently flooded with Rhine water, foreland soil, pasture. Heavy "zavel", clay 22%, org. matter 6%, calcareous, pH 7.9.
2. Hannover, sandy soil from Luneberger Heide, podsol; soil from project Eurosoil, pH 6.2.
3. Schoonebeek, peat soil, pasture. Eastern part of the Netherlands. Org.matter > 95%, pH 5.5

Results are shown in fig. 2; it appears that the adsorption is strongly dependent on the soil type. The results confirm the idea that a major part of the Cr must be precipitated as $\text{Cr}(\text{OH})_3$, the highest leaching is indeed obtained with Cr-anions in the peat soil.

Publications.

Note presented at the 2nd annual meeting on heavy metals in food chain.
Annual report 1972 of the Association EURATOM-ITAL.

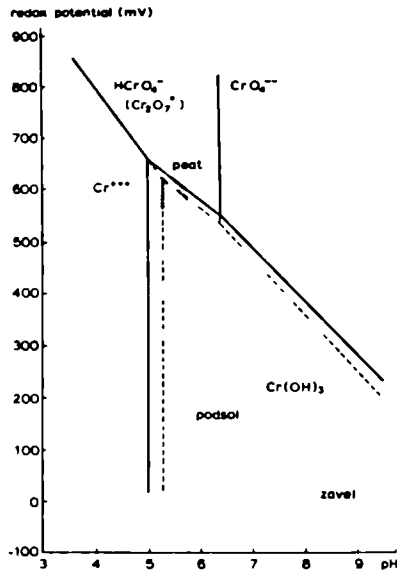


Fig. 1 - Redox potential - pH diagram for Cr.
 Full lines: boundaries 10^{-3} molar (50 ppm).
 Dotted lines: boundaries 10^{-4} molar (5 ppm).
 The regions for the three soils are indicated.

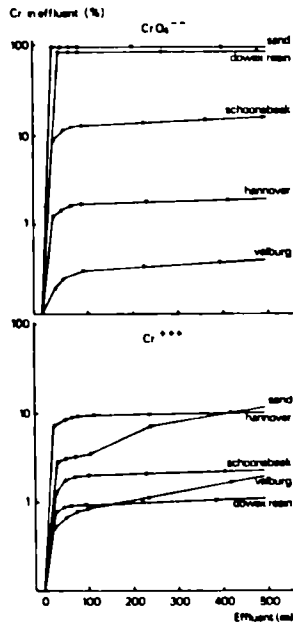


Fig. 2 - Cumulative percentage of Chromium present in the effluent of leaching experiments. The total water of the soils in the columns was approximately 10 ml; the total column volume 20 ml. Influent concentration 5 ppm Cr.

Resultaten van het project No.4.

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra, M. van de Steene.

Titel van het project: Behaviour of mercury and mercury compounds in soils.

Beschrijving van de resultaten:

A mercury monitoring programme was carried out on a number of Dutch soils. Eleven soils were selected, which differed widely in characteristics, location and farming practice. These soils were sampled to a depth of 1 meter and the total mercury content was determined from each 5 cm layer up to a depth of 30 cm and below 30 cm from each 10 cm layer (table 1).

The soils selected can be divided into three groups.

group I: foreland soils from the river Rhine, at Millingen, Valburg and Biesbos respectively. When these sampling sites are arranged according to increasing clay content, c.q. decreasing distance to the North Sea, they show in the upper 20 cm a mercury content of respectively 0.10, 3.3 and 10.4 ppm. Deposition and accumulation of mercury does occur; obviously this mercury stems from the river Rhine, and is strongly absorbed by clay.

group II: soils situated in areas used for bulb culture, at Hillegom and Anna Paulowna Polder. The total mercury content in the upper 20 cm is 0.16 and 0.13 ppm respectively. There is no excessive mercury accumulation, although mercury compounds have been applied as fungicides for many years. When considering, however, the amount of mercury in the 0-50 cm layer these soils contain twice as much mercury as the reference soils. (group III).

group III: soils permanently used as pastures, no profile disturbances occurred for the last 20 years. These series of soils have widely differing soil characteristics and include calcareous and non-calcareous soils, a variation in clay content from 2 to 40% and a peat soil with an organic matter content of more than 95%. These soils can be considered to be references, because no mercury has been introduced. All soils show a mercury content, in the upper 20 cm layer, of approx. 0.09 ppm, except one which is

polder in the Biesbos area reclaimed about 50 years ago.
 So even 50 years ago the river Rhine was already polluted
 with mercury.

B. Transport of some mercury compounds in undisturbed soil columns.
 The compounds studied are: Hg-203 labelled HgCl_2 , CH_3HgCl and Hg^0 ;
 the experiments are carried out on soil from the area for bulb culture;
 sampling site: Hillegom; length of the soil core in the column about
 100 cm. The top soil in this column is labelled homogeneously with
 the compound studied. The artificial rainflux is 24 mm per 24 hours.
 Air is permanently sucked over the column to remove escaping mercury
 vapours and is led through a number of washing bottles containing
 liquids suitable to determine the types of the escaping volatile mercury
 compounds.

According to the present information HgCl_2 does not move under these
 conditions and approx. 1 o/oo of the added mercury has escaped from
 the soil over a period of 3 months.

The mono methyl mercury shows some mobility and during the first months
 of the experiment about 1% escaped from the soil. The experiments with
 metallic Hg have not yet been started.

Table 1 - The mercury content respectively in the 0-20 and 20-100 cm
 layer of Dutch soils.

Group	Location	Hg content ppm	
		0 - 20 cm	20 - 100 cm
I-1	Millingen	0.10	0.01
I-2	Valburg	3.3	0.45
I-3	Biesbos	10.4	2.6
II-4	Hillegom	0.16	0.10
II-5	A.P. Polder	0.13	0.05
III-6	Biesbos	0.35	0.26
III-7	Alkmaar	0.09	0.02
III-8	Hilversum	0.09	0.02
III-9	Amersfoort	0.09	0.01
III-10	A.P. Polder	0.08	0.03
III-11	Schoonebeek	0.07	0.04

Publication :

Annual report of the Association EURATOM-ITAL.

Resultaten van het project No. 5

Hoofd van het team en wetenschappelijke medewerkers:

G. Verfaillie.

Titel van het project: Kinetics of uptake of heavy metal-ions by intact plants.

Beschrijving van de resultaten:

1. Aim

The scope of the research is the knowledge of the mechanisms by which the plant mineral nutrition might be a vector of the pollution by chromium. In 1972, the study has been restricted to the absorption of chromium from nutrient solutions polluted with Cr^{III} -EDTA and with K_2CrO_4 .

2. Materials and methods

9 kinetic runs have been performed with batches of 24 six weeks old rice plants (Oryza sativa L. cv Arborio). The technique and the experimental set-up are the same as those used in project 7 of which project 5 (Radiation protection) is an application. The tracing of the chromium has been done with ^{51}Cr .

3. Uptake from a solution containing Cr^{III} -EDTA

.1 The kinetics

The rate of uptake is extremely low but fits a saturation kinetics. One single mechanism has been found for concentrations ranging from 1.8×10^{-6} M/l up to 10^{-4} M/l Cr^{III} -EDTA. The kinetic parameters based on a Michaelis formalism are:

$$V_m = 8.35 \text{ nM per day and gram fresh root.}$$

$$K_m = 1.85 \times 10^{-5} \text{ M/l}$$

$$(V/C)_m = 0.45 \text{ ml per day and gram fresh root.}$$

2 The distribution

The small amount of chromium taken up is readily transported and distributed all over the entire plant. During a first period of one day the collars and the leaves accumulate the chromium taken up while the roots and the stems behave merely as conductors. Later on, the leaves are saturated, the collars still accumulate at a slowly decreasing rate while the roots and the stems begin to accumulate for themselves.

3.3 The tolerance

No biological disease could be observed on the plants even after a continuous uptake during 5 days with a concentration equal to 10^{-4} M/l $\text{Cr}^{\text{III}}\text{-EDTA}$.

.4 Conclusion

- Chromium chelated with EDTA is taken up extremely slowly but is readily transported all over the plants.
- The absorption seems to be a real physiological uptake.
- The mineral nutrition of plants cannot be considered as a pollution vector for chromium when the latter is chelated with EDTA.

4. Absorption from a solution containing K_2CrO_4

.1 The kinetics

When the initial conditions are only taken into account, the influence of the chromate concentration on the rate of absorption of chromium reveals the existence of 2 mechanisms with a threshold concentration approximating 2×10^{-6} M/l. Both mechanisms are of the saturation type and may be represented by Michaelis curves as in fig. 1.

The kinetic parameters are:

for the low concentration mechanism: $V_m = 2.16 \text{ nM/h.gFR}$
 $K_m = 0.455 \text{ }\mu\text{M/l}$
 $(V/C)_m = 4.75 \text{ ml/h.gFR}$

for the high concentration: $V_m = 34.2 \text{ nM/h.gFR}$
 $K_m = 34.7 \text{ }\mu\text{M/l}$
 $(V/C)_m = 0.98 \text{ ml/h.gFR}$

When the time during which the absorption proceeds, is taken into account, the relation between concentration and absorption rate is completely different as it is shown in fig. 2 a and 2 b. To the contrary of what happens in enzymatic reactions, the saturation factor does not remain constant during the process.

It has also been observed that the absorption of chromate increases the rate of acid consumption.

.2 The distribution

To the contrary of what happens with $\text{Cr}^{\text{III}}\text{-EDTA}$, about 98

percent of the chromium absorbed remains bound to the roots. However, the small translocated amounts are of the same order of magnitude in both cases.

This suggests that the variable saturation factor involved in the absorption process would be the root absorbing surface itself, that should react with the chromate ions. Such a reaction would also involve hydrogen ions (see 4.1).

4.3 Physiological effects

The absorption of chromate is accompanied by strong physiological effects varying with the chromate concentration. At concentrations higher than 2×10^{-5} M/l, the leaves turn yellow and show wilting. Even at a concentration of 5×10^{-7} M/l, a reduction of the transpiration of about 40 percent has been observed.

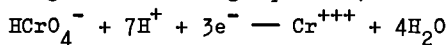
.4 The influence of the pH on the chromium absorption rate.

The initial rate of the chromium absorption at the concentration of 10^{-5} M/l K_2CrO_4 varies linearly with the pH but different parameters are found when the acid consumption is compensated or not. Both linear relations are shown in fig. 3. When the acid consumption is not compensated by a pH-stat, the absorption of $HCrO_4^-$ proceeds against an extra thermodynamic potential due to the continuous displacement of the ionic dissociation equilibria of the diprotic chromic acid. (compare with $H_2PO_4^-$ in project 7 (Application)).

.5 The reduction hypothesis

All the observations reported above suggest that the absorption of chromium is due to the reduction of the chromate by the root surface followed by a major chemical sorption and a minor physiological uptake of the resulting chromic form.

Considering the following equation,



the increase of the acid consumption due to the chromium absorption is explained. Moreover, the linear dependance of the absorption rate upon the pH would imply the linear dependance upon the redox potential.

Indeed:

$$\frac{dVa}{dE} = \frac{dVa}{dpH} ; \frac{dE}{dpH} = \frac{-5.1 \text{ mM/h.gFR per pH unit}}{-0.14 \text{ Volts per pH unit}} = 36.4 \text{ nM/h.gFR.Volt}$$

4.6 Conclusions

- Chromium given as K_2CrO_4 is readily absorbed by the plant roots but only a negligible part of it is translocated to the shoots.
- The absorption would be due to the reduction of the chromate by the root surface.
- Lowering the pH enhances the translocation of the absorbed chromium.
- As far as the aerial parts are concerned, the plant mineral nutrition cannot be considered as a vector of the pollution by chromium, when the latter is in the chromate form.

Publications on the subject in 1972:

G. Verfaillie. Physico-chemical considerations about the use of Hoagland-Arnon I nutrient solution in relation to chromium pollution studies.

(Working group report, April 1972).

G. Verfaillie. The kinetics of chromium absorption by intact rice plants (Oryza sativa L. cv Arborio).

(Working group report, December 1972).

Annual report 1972 of the Association EURATOM-ITAL.

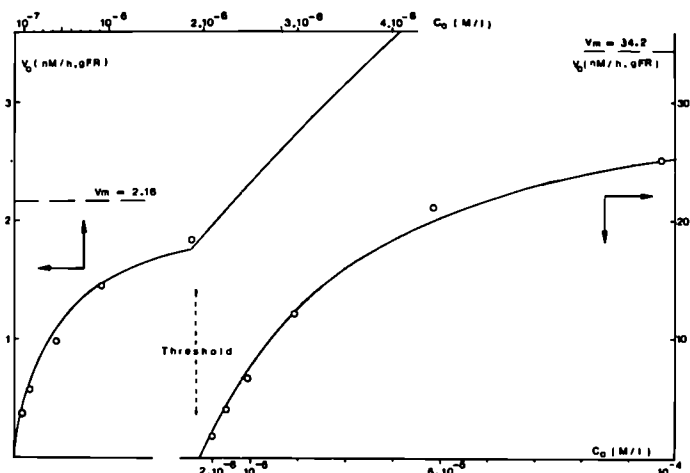


Fig. 1 The initial rate of chromium absorption in relation to the initial chromate concentration.
 V_o = initial rate of chromium absorption (nano-mole per hour and per gram fresh root).
 C_o = initial chromate concentration (mole per liter).
 V_m^o = maximum initial rate of chromium absorption at the saturation of each mechanism.

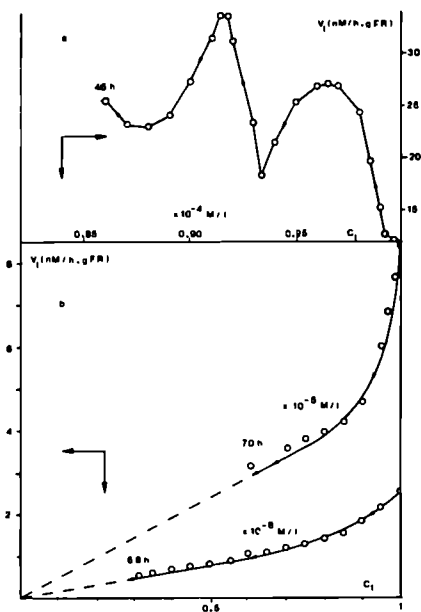


Fig. 2 Variations of the chromium absorption rate with the chromate concentration during a continuous run.
 V_t = time dependent rate of chromium absorption (nano-mole per hour and per gram fresh root).
 C_t = time dependent chromate concentration (mole per liter).
 The arrows along the curves indicate the direction of the concentration scanning during the runs.
 The duration of the runs are written near the end points of the curves.

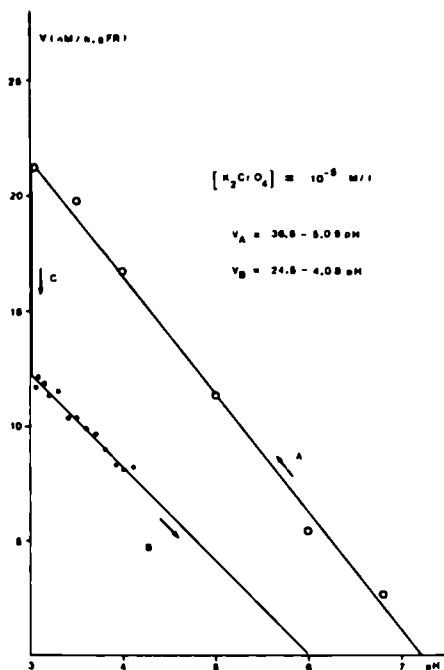


Fig. 3 The influence of the pH on the initial rate of chromium absorption for a chromate concentration equal to 10^{-5} m/l . V = initial rate of chromium absorption (nano-mole per hour and per gram fresh root).
 A = at constant pH (stepwise scanning of the pH levels).
 B = with self-floating pH (continuous scanning of the pH).
 C = stopping of the pH-stationization.
 The arrows along the curves indicate the direction of the pH scanning during the complete run.

Resultaten van het project No. 6.

Hoofd van het team en wetenschappelijke medewerkers:

C. Petit.

Titel van het project: Transport, accumulation and redistribution of heavy metals in intact plants.

Beschrijving van de resultaten:

The scientist in charge of this project, arrived in October 1972.

The final research programme, mainly on cadmium, has been worked out and the first experiments are in progress.

Resultaten van het project - No. 7.

Hoofd van het team en wetenschappelijke medewerkers:

G. Desmet, A. de Ruyter, A. Ringoet.

Titel van het project: Uptake and release of heavy metals by subcellular structures, mainly chloroplasts and mitochondria.

Beschrijving van de resultaten:

A. On chromium uptake by isolated chloroplasts.

This project is part of the collaboration programme on "Heavy metals in the food chain" (Association, Wageningen - Biology, Ispra). Mainly from animal-physiology research it is known that heavy metals frequently form protein-complexes and therefore it was worthwhile to learn more concerning their reaction with plant-membranes from spinach (*Oleracea spinacea* L. cv. Noorman). The isolated chloroplast was chosen as a model.

A few preliminary experiments, considering the chemical form (Cr^{3+} , CrO_4^{2-} , Cr-EDTA), at the highest physiologically acceptable concentration for Cr^{3+} and CrO_4^{2-} (6 ppm) and at 4, 6 and 8 ppm for Cr-EDTA, were made. In all experiments chloroplasts were submitted to a one hour introductory incubation period in calcium solutions of different concentration (membrane permeability). Results in fig. 1a, b show that:

- the uptake of CrO_4^{2-} and Cr^{3+} either reaches an equilibrium exchange after 10 min or is blocked by a self-inhibition mechanism.
- Cr^{3+} -uptake is smaller than the CrO_4^{2-} -uptake. Cr-EDTA absorption, which was negligible at different concentrations and pH, is not represented.
- the different calcium concentrations of the introductory treatment have opposite effects on the uptake of Cr^{3+} and CrO_4^{2-} : higher chromate but lower chromic absorption at increased calcium concentration.

From these preliminary results is concluded that:

- both, negatively and positively charged Cr-ions are taken up by the chloroplasts.
- the negligible uptake of Cr-EDTA is probably due to the dimensions of this complex molecule.

An observed pH-effect (maximum uptake at pH 6 to 7) suggests that Cr^{3+} -ions, resulting from the partial disintegration of the complex, may be responsible for the very small uptake measured.

- there may be some competition between Ca^{2+} and Cr^{3+} for the same binding sites.
- in the case of chromate, its uptake probably compensates to a certain extent for the absorption of the positively charged Ca-ions.
- the calcium concentrations used in the present experiments do not permit any conclusion on the direct influence of this element on chloroplast membrane permeability.

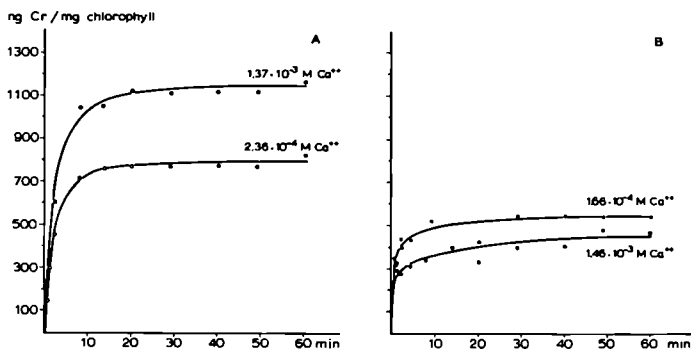


Fig. 1 - Uptake of chromate (a) and Cr^{3+} (b) by isolated spinach chloroplasts after pretreatment with Ca-solutions of different concentration.

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project No. 8

Hoofd van het team en wetenschappelijke medewerkers:

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

Titel van het project: Primary radiation effects in inert and biological material.

Beschrijving van de resultaten:

The molecular theory

This new theory is based on the damage of a double molecular target and gives an equation for cell survival

$$S = \frac{-aD}{e} - \frac{bD^2}{e}$$

where the coefficients a and b contain parameters which take into account the various repair processes and the effect of different types of radiation.

The theory has been successfully used to analyse the variation of radiation sensitivity in the cell cycle. The results of this analysis show that the equation can be fitted to the survival of synchronized cells irradiated in different phases of the cell cycle (fig. 1) and that the coefficients 'a' and 'b' show a consistent variation through the cell cycle independent of the type of cell. The coefficient 'a' shows a dip in the S phase (fig. 2) and this is explained on the basis of the double molecular target being the two strands of the DNA double helix which partially opens during the S phase.

The theory contains implicitly, through repair mechanisms and the damage of a double target, a dose rate effect. Theoretically the dose rate effect should be revealed in a changing 'b' coefficient, 'b' decreasing as the dose rate decreases. It predicts that for survival curves made at constant dose rates with the same cells and radiation the 'a' coefficient should remain constant and the 'b' coefficient should change. Analyses of published work have confirmed this theoretical expectation and the dose rate effect is explained on the basis of enzymatic repair of DNA single strand breaks. As the modern concept of a chromosome is a long DNA-double helix 'backbone' with attached protein and ribosomes, a chromosome aberration may be considered to arise from a break in the DNA double helix 'backbone', that is a DNA double strand break. This

DNA double strand break is the damage which is the basis of the molecular theory of cell survival and consequently the same process of radiation induced double strand breakage has been applied to chromosome aberrations. The result is a new theory of chromosome aberrations which gives the equation

$$Y = \alpha D + \beta D^2$$

for all chromosome aberrations. The theory differs from the classical and exchange theories currently in use in that each chromosome aberration is based on one chromosome break plus incorrect rejoining instead of two chromosome breaks plus exchange.

Polymethylmethacrylate (PMMA)

The results of a study of the dependence of the OD-dose relationship in PMMA on irradiation at temperatures from -196°C to 20°C have been analysed.

The OD-dose relationships are different at different temperatures but each curve can be fitted by an equation based on a first order radical destruction process. The coefficient of radical destruction is also temperature dependent. The most likely explanation is: from -196°C to -100°C the curve is determined by the production and destruction of a radical or ion giving the singlet ESR spectrum and the activation energy of the destruction process is 0.0045 eV; from -100°C to 20°C the curve is determined by the production and destruction of the radical giving the nine line ESR spectrum and the activation energy of this destruction process is 0.03 eV.

Publications:

Chadwick, K.H. The effect of light exposure on the optical density of irradiated clear polymethyl methacrylate. Phys. Med. Biol. 17 88-93. 1972.

Chadwick, K.H. A discussion of the role of free radicals in the biological effect of different LET radiation. Proc. 3rd Euratom symposium on Microdosimetry. Stresa October 1971. 253-265. 1972. EUR 4810 d-f-e.

Chadwick, K.H. A comparison of the radical dose relationship in clear polymethyl methacrylate following gamma and fast neutron irradiation. Proc. 3rd Euratom Symposium on Microdosimetry. Stresa October 1971. 237-252. 1972. EUR 4810 d-f-e.

ten Bosch, J.J., W.F. Verhelst and K.H. Chadwick, Relation between free radicals and ultra-violet absorption in polymethyl methacrylate. J. of Polymer Sci. Part A-1. 10. 1679-1685. 1972.

Annual Report 1972 of the Association EURATOM-ITAL.

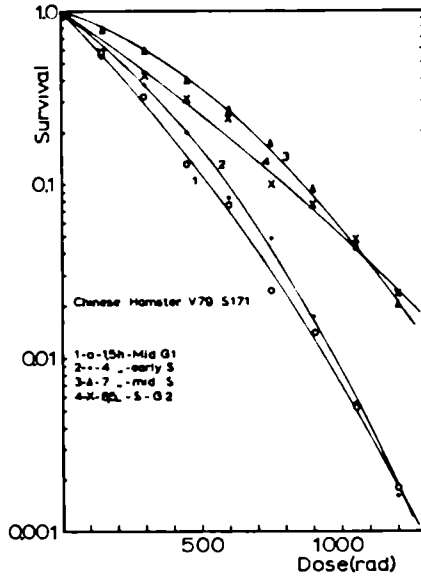


Fig. 1. Survival curves for synchronised cells at different stages of the cell cycle fitted by the equation

$$S = e^{-aD} \cdot e^{-bD^2}$$

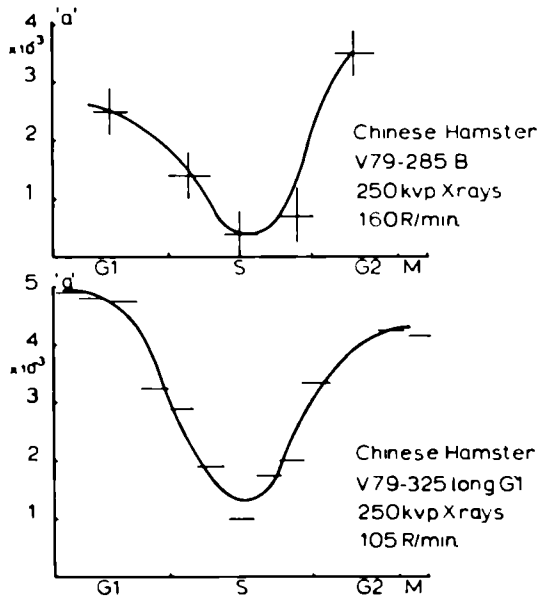


Fig. 2. Variation of the coefficient 'a', in the cell cycle for two different cell types.

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:

Thermoluminescence measurements on BeO irradiated at liquid nitrogen temperature

BeO shows a supralinear TL response with increasing dose, as does LiF. Both materials exhibit glow peaks in the temperature range from - 196°C up till 400°C. According to literature, the degree of supralinearity for LiF is a linear function of the cube of the absolute peak temperature.

The measurement on BeO are aimed 1) to find out if this T^3 relationship is a general one and also holds for the supralinearity of BeO and 2) to get an insight in the mechanism of the supralinearity of BeO.

BeO discs were irradiated at - 196°C and read out in an atmosphere of He up to 70°C. At this temperature the discs were replaced to another read out apparatus for recording the TL glow curves up to 350°C.

Using a heating rate of 20°C/min peaks were recorded at a heater temperature of - 113, - 95, - 12, + 40, + 173 and + 267°C.

The dose response curves indicate that a T^3 relationship is not present in BeO.

Measurements of emission spectra and the use of special filtration may lead to reduction of the disturbing supralinearity effect in BeO.

Publications:

Leenhouts, H.P. Another failure of a commercial X-ray shutter system. Health Phys. 22. 413-414. 1972.

Puite, K.J., D.L.J.M. Crebolder, J.J. Broerse, and B. Hogeweg. Intercomparisons of absorbed dose and dose distribution for X-irradiations using mailed LiF thermoluminescent dosimeters. Phys. Med. Biol. 17. 390-399. 1972.

Puite, K.J. and J. Arends. Trapping centers in CaF₂ Mn from TL and TSEE measurements on undoped and doped CaF₂ samples. Proc. 3rd Cong. on Luminescence Dosimetry Risø, October 1971. Report 249. part 2. 680-691. 1972.

Annual Report 1972 of the Association EURATOM-ITAL.

by H.LAUDELOUT

List of scientists having contributed to this report :

Dr.R.Van Bladel, Dr.Tang Van Hai, Dr.Gloria Gaviria, Mr.Fageria.

I. Ion Movement and Exchange in Soils.

The modelling of the movement of ions or molecules in the soil profile require that several parameters are known fairly accurately whether it is contemplated to study the movement of radionuclides, plant nutrients or biocides.

If the model is to be of sufficient generality, the relationships between the parameters measured and the properties of the system studied should be as fundamental as possible.

One of the important factors in this respect is the influence of the salt concentration in the soil solution on the exchange affinities of various ions. It has been shown that the factor of importance in this respect is the hydration numbers difference of the ions involved in the exchange.

Ion exchange equilibria for four pairs of singly charged ions in a montmorillonite clay were carried out at different total normalities of the equilibrium solution at 25°C. Only the trace region for the preferred ions was investigated. The effect of solvent activity a_w on the selectivity coefficient K_c was established. In each case the expected linear relationship between $\ln K_c$ and $\ln a_w$ was observed. The values for the differences of the ion hydration numbers, $n_w^A - n_w^B$, calculated from this purely thermodynamic relationship, were consistent and were comparable with values that could be deduced from basal spacings or anion exclusion volume data.

H.Laudelout, R.Van Bladel and J.Robeyns : "Hydration of Cations Adsorbed on a Clay Surface from the Effect of Water Activity on Ion Exchange Selectivity". Soil Science Society of America Proceedings, 1972, 36, 30-34.

An additional reference on a related subject has appeared during 1972. R.Van Bladel, Gl.Gaviria and H.Laudelout : "A Comparison of the Thermodynamic Double Layer Theory and Empirical Studies of the Na-Ca Exchange Equilibria in Clay Water Systems". Proceedings International Clay Conference, Madrid, 1972, II, 15-30.

Another important aspect influencing ion movement in soils is the tortuosity of the pore systems (see for instance A.Cremers and H.Laudelout : "Conductivité électrique des gels argileux et anisométrie de leurs éléments". J.Chim.Phys., 1965, 10, 1155-1162.

Considerable attention has been devoted to this problem during the last year (Gaviria Gl. : "Influencia de la hidratacion de las arcillas sobre los fenomenos de transporte en los geles arcillosos", Thesis submitted for a doctorate at the University of Louvain, December 1972).

The results from the work carried out may be summarized as follows : if the electrical conductivity of a soil paste K_g is related to the electrical conductivity of the salt solution filling its pores K_s , a very good agreement can be found between the experimental results and the predictions of Maxwell's theory expressed by Fricke's formula :

$$K_s - K_g = \frac{(K_s - K_i) \rho R}{1 + \rho R}$$

with
$$\rho = \frac{1}{3} \frac{\rho'}{1 - \rho'}$$

where ρ' is the volume fraction of the solid phase and R is given by :

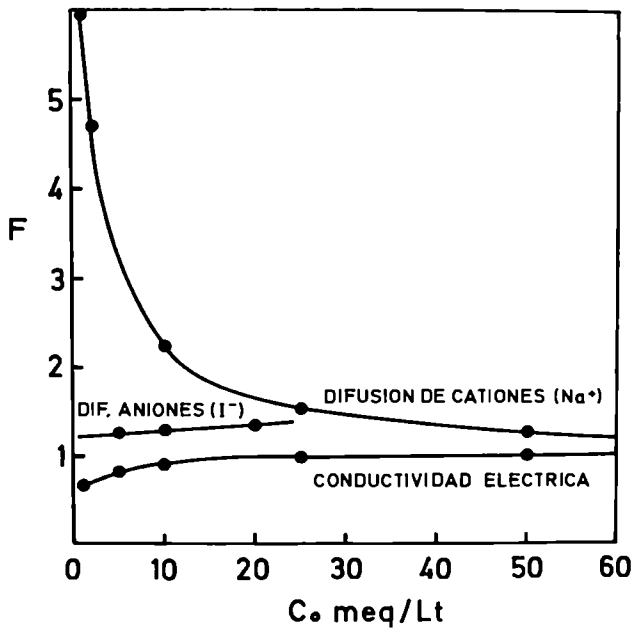
$$R = \frac{2}{1 + \left(\frac{K_i}{K_s} - 1 \right) \frac{M}{2}} + \frac{1}{1 + \left(\frac{K_i}{K_s} - 1 \right) (1 - M)}$$

where K_i is the conductivity of the solid phase and M is a factor related to the shape of the particles.

We have shown that this agreement demonstrated earlier by Thomas and Cremers (J.Phys.Chem., 1970, 74, 1072: "Electrical Conductivity of Suspensions of Conducting Colloidal Particles") is illusory and due to the arbitrary adjustment of three independent parameters.

If the hydration of the particles is measured by negative adsorption techniques and the hydration numbers used for calculating the volume fraction of the hydrated solid phase the agreement between theoretical predictions and experimental results is shown to be restricted to a fairly low range of volume fractions. It is thus more suitable to use the empirically determined formation factor defined as the ratio of the rate of transport in the solution to that in the solution than theoretical relationships which obviously do not apply in soil systems.

The obstruction effect of clay-water systems becomes largely independent of the techniques used for its measurement as soon as the salt concentration becomes high enough as shown in the figure.



Formation factor measured by three different techniques in clay-water systems as a function of electrolyte concentration.

II. Uptake of solutes by plants from a dilute environment.

Most, if not all of the physiological studies on mineral nutrients uptake by plants have been carried out on relatively concentrated nutrient solutions. Several problems relating to the radio contamination of the environment (and generally to the mineral nutrition of plants growing on soils of low fertility level) demand a better knowledge of processus of uptake from very dilute solutions. Studies on the uptake of phosphate, as a model-element, by the rice plant from extremely dilute solutions have been initiated several years ago : (Tang Van Hai and H.Laudelout . Soil Sci., 101, 408-417 (1966)).

The emphasis was placed from the start on obtaining accurate experimental data and modelling the phenomenon. This research has been carried out in close cooperation between the Association EURATOM-ITAL and the Louvain group since several years. This is shown by the following publications which appeared during 1972 :

- Tang Van Hai, N.K.Fageria and H.Laudelout : "Root Electrical Potential and Phosphate Uptake of Rice as determined by pH".
Reprint from "Isotopes and Radiation in Soil-Plant Relationships including Forestry", IAEA, Vienna, 1972, 81-86.
- Alagar Swamy, Tang Van Hai, M.Frissel, H.Laudelout, A.Ringoet :
"Analysis of response curves to increased phosphate concentration in intact rice and groundnut plants".
Reprint from "Isotopes and Radiation in Soil-Plant Relationships including Forestry", IAEA, Vienna, 1972, 87-94.

Most of the work done in 1972 has been concerned with the influence of other ions on the uptake kinetics of a given ion from a very dilute solution. This was the physiological counterpart of the purely physico-chemical study carried out previously (Tang Van Hai & H.Laudelout : "Phosphate Uptake and Root Electrical Potential". Journal of Experimental Botany, 1971, 22, 830-836.

Rice plants (*Oryza sativa* L var.IR8) were grown in dilute nutrient solutions with varying concentrations of calcium, phosphorus, potassium and magnesium.

1. Ion Interaction

The uptake of phosphorus was independent of calcium concentrations in the range of 6 μM to 748 μM calcium. Absorption of potassium and magnesium was stimulated in the presence of calcium ions at low calcium concentrations. But potassium and magnesium absorptions were inhibited at higher calcium concentrations.

Rate of potassium absorption as well as tissue potassium concentration were independent of phosphorus concentration.

At low concentration of phosphorus, the rate of uptake as well as the total magnesium content of the plants increased but at higher concentrations of phosphorus, magnesium uptake was depressed.

The effect was more pronounced for tissue concentration. Increasing concentrations of potassium in the nutrient solution depressed phosphorus, calcium, and magnesium absorption rate as well as concentrations of these elements in the plants. But under higher concentrations this effect was diminished.

Magnesium at high concentrations caused decreases in potassium, phosphorus and calcium contents of the plants tissue. A similar trend was recorded in the rate of absorption of potassium and phosphorus.

2. Ion uptake at different growth stages.

Rice plants showed a marked response in both rate and, of course, total ion accumulation with respect to age.

Potassium, phosphorus and magnesium uptake rates increased up to 75 days and then declined. The concentrations of calcium, phosphorus, potassium and magnesium in the plants gradually declined from the seedling stage till maturity.

Université Catholique de Louvain

N° 096-72-1 BIO B

A. GOFFEAU

Transport des radionuclides par les structures membranaires biologiques.

L'utilisation de mitochondries fonctionnelles de levures dont l'isolement avait été mis au point en 1971, a permis de réaliser d'intéressants progrès en 1972. En particulier, une stimulation par le strontium de la respiration mitochondriale a été observée. Cet effet, particulièrement marqué lors de l'oxydation du NADH, nécessite des concentrations de 1 à 10 mM de SrCl_2 . L'étude détaillée des nombreux paramètres susceptibles de modifier la stimulation de la respiration par le strontium a montré que celle-ci ne peut être identifiée à aucun effet décrit précédemment. L'action du strontium semble s'exercer à un niveau très primaire, antérieur à celui auquel agissent les agents découplants de l'oxydation phosphorylante. On peut espérer que l'étude plus détaillée de l'interférence du strontium avec l'équilibre ionique mitochondrial fournira des données intéressantes sur le rôle de celui-ci dans l'oxydation phosphorylante.

Des progrès notables ont également été réalisés en ce qui concerne l'étude du mode d'action d'agents chélateurs des terres rares et des métaux lourds: les dithiocarbamates. Ces composés utilisés en pharmacie et dans certaines industries inhibent préférentiellement la succinate déhydrogénase mitochondriale de levure. Un parallélisme étroit a été observé entre son action aux niveaux de l'oxydation et de la perméation des anions dicarboxyliques. Cette étude a permis d'avancer l'hypothèse d'une intervention directe de la succinate déhydrogénase dans le transport des dicarboxylates à travers la membrane mitochondriale de levure.

D'autre part, l'obtention par irradiation aux rayons X et la caractérisation biochimique et génétique de mutants de levure, déficients dans leurs fonctions mitochondriales, a fortement progressé. L'état actuel de ces travaux permet d'espérer l'utilisation prochaine de ces outils génétiques dans l'étude des phénomènes de transport des radionuclides par les mitochondries isolées.

Enfin, signalons la mise en place d'un nouvel équipement de microscopie électronique et le perfectionnement de diverses techniques morphologiques et biochimiques nécessaires à l'étude du rôle des membranes plasmiques de levure dans le transport des radionuclides. Ces travaux encore préliminaires ont permis l'observation de structures intéressantes constituées par l'invagination de la membrane plasmique de levure.

Publications

1. M. BRIQUET - Action du Ziram sur les fonctions mitochondriales.
Arch. Intern. Physiol. Biochim. 80, 1, 181-182, 1972
2. A. GOFFEAU, Y. LANDRY, A.M. COLSON et F. FOURY - Dual control of synthesis of oligomycin-sensitive ATPase in a "petite-negative" yeast.
Abstract Communications Meetings Federation European Biochemical Societies, 8, 610, 1972.
3. A. GOFFEAU, A.M. COLSON, Y. LANDRY and F. FOURY - Modification of mitochondrial ATPase in chromosomal respiratory-deficient mutants of a "petite-negative" yeast : Schizosaccharomyces pombe 972h⁻.
Biochem. Biophys. Res. Comm., 48, 1448-1454, 1972.
4. F. FOURY and A. GOFFEAU - Glucose superrepressed and derepressed respiratory mutants in a "petite-negative" yeast : Schizosaccharomyces pombe 972h⁻:
Biochem. Biophys. Res. Comm. 48, 153-160, 1972.
5. Y. LANDRY et A. GOFFEAU - Isolement et caractérisation du complexe ATPasique mitochondrial sensible à l'oligomycine chez la souche sauvage et des mutants respiratoires d'une levure "petite-négative" Schizosaccharomyces pombe.
Arch. Intern. Physiol. Biochim. 80 (3), 604-606, 1972.
6. A.M. COLSON, C. COLSON and A. GOFFEAU - Systems for membrane alteration: genetic perturbations of mitochondria in a "petite-negative" yeast.
Methods in Enzymology, 1973,(sous presse).
7. F. FOURY and A. GOFFEAU - Combination of 2-deoxyglucose and snail gut enzyme treatments for spheroplast preparation in Schizosaccharomyces pombe.
J. Gen. Microb: 74, 1973 (sous presse).

Projet N° I

A. GOFFEAU, M. BRIQUET, A. SOUCHAY, F. FOURY, Y. LANDRY

Transport des radionuclides par les mitochondries isolées de levure.

1. Action du strontium sur les mitochondries isolées de levure (A. SOUCHAY, A. GOFFEAU, M. BRIQUET).

La présence de 1 à 10 mM de SrCl_2 stimule la respiration de mitochondries isolées de Saccharomyces cerevisiae. L'ampleur de cette stimulation varie selon la nature du substrat oxydé. Mesurée à son pH optimum (5.8 à 6.5) elle est de 25% pour le citrate et le succinate, 60% pour l'éthanol et de 150% pour le NADH. A l'exception du beryllium, tous les cations de la série des alcalino-terreux ont un effet stimulateur semblable. Par contre, les cations divalents de la série du fer préviennent de façon non compétitive l'effet du SrCl_2 . L'augmentation de perméabilité aux protons de la membrane mitochondriale par les découplants de l'oxydation phosphorylante ou le vieillissement à 0°C des mitochondries isolées, favorisent l'action stimulatrice du SrCl_2 . Le SrCl_2 lui-même n'exerce pas d'action découplante puisqu'il permet et généralement favorise la phosphorylation de l'ADP. L'inhibition par l'oligomycine de la phosphorylation de l'ADP et de l'ATPase mitochondriale n'interfère pas avec la stimulation de respiration par le strontium. Par contre, en conditions non phosphorylantes, l'ADP accentue l'effet du SrCl_2 tandis que le phosphate contrecarre celui-ci. Aux concentrations de l'ordre de 100 μM , le chlorure de lanthane ainsi que le rouge de ruthenium préviennent toute stimulation de respiration causée par les alcalino-terreux aussi bien que par les agents découplants ou par la phosphorylation couplée de l'ADP.

Les caractéristiques de ces effets sont totalement différentes de celles décrites précédemment dans la littérature. Les résultats obtenus sont difficilement interprétables dans le cadre des théories impliquant un transport de cations causé par un intermédiaire chimique de haut contenu énergétique hydrolysé par les agents découplants. Par contre, les faits expérimentaux pourraient être interprétés selon la théorie dite chimio-osmotique. Dans ce cas, la stimulation de respiration serait le résultat d'une diminution de la composante ionique du potentiel de membrane et il faudrait admettre que cette composante ionique puisse être modifiée indépendamment du gradient de pH aboli par les agents découplants.

2. Le Ziram, inhibiteur préférentiel de l'oxydation et de la perméation du succinate chez les mitochondries de levure (M. BRIQUET).

Le diméthylldithiocarbamate de zinc (Ziram) inhibe préférentiellement l'oxydation du succinate par des mitochondries isolées de levure (Publication n° 1). A la concentration de 30 μ M, le Ziram inhibe de 77% l'oxydation du succinate stimulée par la phosphorylation couplée de l'ADP chez des mitochondries intactes. L'oxydation et le contrôle respiratoire inhibés peuvent être rétablis par l'addition d'autres substrats tels que l'éthanol, le NADH ou l' α -glycerophosphate. En l'absence de barrière de perméabilité, les activités succinate déhydrogénases de particules submitochondriales et de l'enzyme solubilisée sont inhibées de 30 à 50% par 30 μ M de Ziram. A cette même concentration, la perméation passive du succinate, mesurée en l'absence de respiration, est fortement ralentie. La perméabilité d'autres substrats tels que l' α -glycerophosphate, le citrate et l' α -cetoglutarate n'est pas affectée, par contre, celle du malate est également inhibée. Le parallélisme observé dans la spécificité d'action du Ziram sur l'oxydation du succinate et sur sa perméation suggère qu'une relation étroite existe entre la fonction catalytique de la succinate déhydrogénase et le transport des anions dicarboxyliques.

3. Obtention par irradiation et caractérisation de mutants de levures déficients dans la respiration (A. GOFFEAU, Y. LANDRY, F. FOURY).

Dans le but d'étudier comment l'altération génétique de la membrane mitochondriale peut modifier le transport des radionuclides, une série de mutants pleiotropiques de levure, déficients à la fois dans les cytochromes a et b et dans l'ATPase mitochondriale ont été caractérisés biochimiquement et génétiquement (publications 2 à 6). Il résulte de l'étude approfondie du mutant M126, obtenu par irradiation aux rayons X, qu'une mutation dans un seul gène nucléaire est à l'origine de multiples déficiences identiques à celles obtenues par altérations du DNA mitochondrial. Il semble donc que la mutation nucléaire affecte un mécanisme d'assemblage de la membrane mitochondriale, contrôlé à la fois par le DNA mitochondrial et certains gènes nucléaires.

Plusieurs méthodes originales d'isolement de mitochondries intactes de ces mutants ont été mises au point mais leur utilisation reste délicate (Publication n° 7). Une nouvelle technique utilisant une enzyme isolée d'Arthro-bacter luteus a donné récemment des résultats prometteurs.

Projet N° II

A. GOFFEAU, E. MRENA et J. DELHEZ

Etude du rôle de la membrane plasmique dans le transport des radionuclides par les cellules de levure.

Cette étude est encore dans un état préliminaire de mise au point technique.

Le professeur A. CLAUDE avec lequel nous collaborons dans ce projet a installé un nouvel équipement de microscopie électronique dans des locaux adjacents aux nôtres. L'amélioration des techniques de fixation et d'observation en microscopie électronique de la levure Saccharomyces cerevisiae a fait l'objet de plusieurs expériences. L'état actuel de ces techniques a permis l'observation d'importantes invaginations de la membrane plasmique. Ces structures contiennent des substances de nature diverse : membranes, protéines et granules. Par ailleurs, J. DELHEZ a détecté dans des fractions membranaires obtenues après broyage des cellules, un marqueur probable de la membrane plasmique: l'AMPase. Une fraction membranaire contenant cette activité a pu être débarassée de contaminants mitochondriaux par centrifugation en gradient de saccharose.

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)

Laboratory for Molecular Biology, State University of Leiden, The Netherlands
(in collaboration with the Medical Biological Laboratory TNO, Rijswijk 2100).

Title: STUDY ON THE MECHANISM OF MUTATION IN ANIMAL AND
HUMAN CELLS

Summary:

Mammalian cells have the ability to repair damage induced in their DNA by irradiation or chemical compounds. The molecular mechanisms of these repair processes are largely unknown. In the following report results are presented of studies on repair processes occurring in mammalian cells cultivated in vitro. After irradiation with ultraviolet light. "Repair replication" is a characteristic reaction of the cell, after irradiation; methods to measure repair replication are presented and data on the kinetics of this reaction were obtained.

In this study also cells derived from Xeroderma pigmentosum patients were included. These cells are sensitive to UV light and show a reduced amount of repair replication after irradiation with UV. Studies on damage caused by alkylating agents are also included in this study.

It was shown that normal cells contain an enzyme which specifically reacts with UV irradiated DNA. To gain more insight into the molecular nature of repair processes it is of course important to study enzymes which are involved in these processes. Techniques were set up for the separation of endonucleases.

Using man/Chinese hamster somatic cell hybrids several linkage groups of man were established and moreover several of the studied enzyme loci have been assigned to a chromosome. Hybrid clones isolated from fusion between lymphocytes with a 3/X translocation and Chinese hamster cells gave information about the position of some X-linked loci within the X-chromosome. Genetic transformation by means of homologous wild type DNA was performed in Chinese hamster temperature-sensitive mutant cells.

Human peripheral blood lymphocytes were irradiated in vitro with 250 kV X-rays, 15 MeV neutrons and 18 MeV electrons. Cells were examined at their first mitosis in culture for chromosome aberrations. Analyses of the results was restricted to the number of dicentric chromosomes and centric rings. In the X-ray experiments, the influence of PHA stimulation time (30 min before, 2 and 16 hours after irradiation), temperature during irradiation (room temperature or 37°C) and irradiation of whole blood or of blood in culture medium on aberration frequency was tested.

Project No. A 1.1.

Title: The induction of chromosome aberrations by chemical and physical agents and the automation of the scoring of these abnormalities

Research workers: Drs. H. Heering, Prof. Dr. D. Bootsma, Drs. W. G. Burgerhout, Dr. S. Bacchetti

Progress report:

The dose effect relationship after irradiation with 250 kV, 15 mA X-rays, 8 MeV electrons and 15 MeV neutrons has been determined. X-rays and electrons were equally effective in aberration induction. Neutrons were significantly more effective than these both; the difference was about a factor two.

There was no significant difference in the induction of chromosome aberrations when the stimulation time of PHA was 30 min before or 16 hours after irradiation. There was also no difference in aberrations when the experiments were done in room temperature or at 37°C. In the cases of the whole blood irradiation experiments or of blood in culture medium during irradiation, one result was not significantly different from its control while in the other experiment there was a slightly significant shift towards lower aberration frequencies.

A staining technique for human chromosomes (e.g. atebriane and ASG) has been developed good enough for determination of aberrations and translocations. The techniques are not good enough for automation of the scoring of the abnormalities of the whole karyograms.

Project No. A 1.2

Title: Repair of radiation damage in mammalian cells

Research workers: Dr. G. Veldhuisen, Dr. P.H.M. Lohman, Drs. W.J. Kleijer,
Dr. S. Bacchetti, Dr. R.A. Oosterbaan, Dr. P.H. Pouwels, Prof. Dr. D. Bootsma

Progress report:

Study of the repair of damage of DNA in mammalian cells was carried out along the following lines:

1) Measurement of repair replication in mammalian cells after UV irradiation.

A new technique was introduced to measure repair replication. It consists of isopycnic centrifugation in sodium iodide (NaI) gradients containing EtBr. With this technique several data on repair replication were collected:

- a) study of repair in UV sensitive cells. Normal cells show repair replication when they are irradiated with UV light. Cells from patients with Xeroderma pigmentosum (XP) show a decreased amount of repair replication after UV irradiation. The rate of repair replication differs in the different XP lines isolated: cells from De Sanctis-Cacchione (DSC) patients (a severe case of XP) are completely negative in repair; cells from other XP patients (so-called classic XP) were classified in a group with a low rate of repair (5-10% of the control) and a group with an intermediate rate of repair (20-70% of the control). All these repair rates are genetically determined. It was shown that fusion of DSC-cells with classic XP-cells results in the formation of heterokaryons which show a normal rate of repair replication. So at least two genes are involved in the process of repair replication.
- b) studies on the kinetics of repair replication were performed using several UV doses and measuring repair at different times after irradiation.
- c) the NaI technique for measuring repair replication was also used to try to localize the genes responsible for repair. For this study interspecies hybrids between men and Chinese hamster cells were used. ^{*} It was not possible to connect a "repair gene" with one of the known enzyme markers.

2) Study of excision of thymine dimers. Normal cells have excised about 50% of their dimers after 24 h incubation after UV irradiation. The kinetics of disappearance of dimers from DNA (measured with chromatographic techniques) is comparable with the degree of repair replication at several times after irradiation. Another way to measure the number of thymine dimers present in DNA was introduced.

* see also project 71-14

In this case the extracted DNA was incubated with the enzyme UV-specific endonuclease and analysed in alkaline sucrose gradients. Excision of thymine dimers does not occur or to a much lower extent in XP cells.

Occurrence of single-strand breaks in DNA during repair of UV damage could not be detected with the technique used (sedimentation in alkaline sucrose gradients, in which 0.2 breaks in a DNA molecule of 10^8 daltons was the limit of detection). The possibility was examined that breaks do occur during excision-repair, but are closed very fast by subsequent reactions, so that they escape detection. Therefore inhibitors were added to the cells immediately after UV irradiation, inhibitors which were known to block the repair of X-ray induced breaks (e.g. EDTA, KCN, DNP, iodoacetate and crystalviolet). With none of these compounds accumulation of the presumed "UV breaks" could be shown.

3) Damage by alkylating agents. Incubation of cells with MMS (10^{-4} M) and EMS (5×10^{-3} M) leads to the induction of breaks in DNA strands as was shown after analysis in alkaline sucrose gradients. Preliminary experiments showed that prolonged incubation after the removal of the alkylating agent resulted in repair of the induced damage.

4) Study of repair enzymes in mammalian cells. An enzyme activity has been detected which specifically introduces breaks in UV irradiated DNA (measured in alkaline sucrose gradients). UV irradiated DNA, incubated with photo-reactivating enzyme was degraded to the same extent, so probably another damage than the thymine dimer is recognized by this enzyme. The activity is also present in extracts of XP cells. From preliminary experiments it was concluded that extracts from human cells also contain an enzymatic activity that acts on lesions produced in DNA by ionizing radiation.

Biological reactivation of UV irradiated Φ X-RF DNA with extracts of normal human cells met with failure.

Endonucleases in cell extracts were separated by means of electrophoresis in DNA-polyacrylamide gels. The sensitivity of this technique was enhanced by localizing the enzyme bands with fluorescence measurements.

Project No. A 1.3

Title: Genetic studies on in vitro cultured human and mammalian cells

Research workers: Dr.A. Westerveld, Drs.H. van Someren

Progress report:

Hybrid cell lines obtained after fusion of Chinese hamster cells with human fibroblasts or lymphocytes show a preferential loss of human chromosomes. Since most of the homologous enzymes of man and hamster are electrophoretically distinguishable it is possible to establish linkage groups. The association of genetic markers can be established by their simultaneous retention or loss; the presence or absence of specific phenotypes and specific chromosomes presumes the linkage of a particular gene to a particular chromosome.

Cultured cells of normal persons are able to repair radiation damage (both UV and ionizing radiation). Since Chinese hamster cells have about 20 per cent of the repair replication activity compared with human cells, repair replication can be used as a marker in man/Chinese hamster somatic cell hybrids. Until now no evidence is present that the "repair" locus (loci) is linked with the loci coding for the 30 tested enzymes.

The evidence that in man the locus for glutamic-pyruvic transaminase B (GPT B) is linked with the loci coding for lactic dehydrogenase B (LDH B) and peptidase B (pep B) and the linkage between the GPT C and LDH A loci has been extended. It was shown that in man besides the loci coding for 6-phosphogluconate dehydrogenase (6 PGD) and phosphoglucomutase 1 (PGM 1) also the locus for pep C is situated on the same chromosome.

The expression of the HL-A histocompatibility antigens in man/Chinese hamster hybrid cells has been compared with the absence or presence of 21 independent loci coding for human enzymes. No clear evidence until now is present that the HL-A locus is linked with one or more loci coding for the tested enzymes. An electrophoretic separation between human and Chinese hamster homologous enzymes has been developed for peptidase C, nucleoside phosphorylase (NP) and pyruvate kinase (PK).

In collaboration with Dr.P. Pearson (Dept. of Anthropogenetics, University of Leiden) and Dr.A. Jongasma (Dept. of Cell Biology, Medical Faculty Rotterdam), the following loci have been assigned to a chromosome: 6 PGD, PGM 1 Pep C to chromosome 1; NADP dependent cytoplasmic malate dehydrogenase to 6; LDH A and GPT C to 11; LDH B, GPT B and Pep B to 12; indophenol oxidase B

to 21; hypoxanthine-guanine phosphoribosyl transferase (HGPRT), glucose-6-phosphate dehydrogenase (G6PD), 3-phosphoglycerate kinase (PGK), and α -galactosidase (α -Gal) to X.

To determine the position of the loci for HGPRT, G6PD, PGK and α -Gal within the X-chromosome, lymphocytes from two people having a 3/X translocation, were fused with Chinese hamster cells. The hybrid clones isolated from these fusions were tested for the presence or absence for HGPRT, G6PD, PGK and α -Gal. Preliminary results showed that the structural gene for G6PD is located on the terminal part of the long arm of the X-chromosome. The locus (loci) for α -Gal is segregating independently from the loci for G6PD, PGK and HGPRT.

Genetic transformation by means of homologous wild type DNA was studied in Chinese hamster temperature-sensitive mutant cells. Pretreatment of the recipient cells, prior to addition of transforming DNA, with DEAE-dextrane with presence of high concentration of Ca^{++} gave the best results (up to 10 or 15 times the control). The transformation frequency has been determined under various experimental conditions in order to obtain optimal results. This work is still in progress.

Laboratory for Molecular Genetics, State University Leiden, The Netherlands
(in collaboration with the Medical Biological Laboratory TNO, Rijswijk 2100)

Title: THE IDENTIFICATION OF ENZYMES AND GENES WHICH ARE
INVOLVED IN THE REPAIR OF RADIATION DAMAGE IN BACTERIA

Summary:

The study of repair processes in bacteria form an important source of information on repair processes in general. Under investigation are the processes in *E. coli* and *Micrococcus luteus* which are able to repair damage caused by irradiation with UV- and X-rays.

The mechanism of binding of the ATP-dependent nuclease (an important enzyme in the processes for repair of X-ray damage and recombination) to DNA was studied in detail. The enzyme (purified from *M. luteus*) binds preferentially to a linear double stranded DNA (without or with very short sticky ends) but not with single-stranded DNA or circular double-stranded DNA. ATP is not required for the binding and two enzyme molecules only will bind to each linear duplex.

The expression of the action of the enzyme in recA mutants of *E. coli* was studied. Evidence has been obtained now that the apparent higher ATP-dependent nuclease content of recA over rec⁺ strains must be ascribed to a difference in the state of the DNA in these strains. RecA strains contain a high amount of fragmented DNA which is unable to inhibit the enzyme.

Also rorA mutants show a higher ATP-nuclease activity than ror⁺ strains. The rorA mutation maps inside or very near the recBC locus, the structural gene for the ATP-dependent nuclease. The enzyme has been purified both from rorA and ror⁺ cells and a comparative study is in progress.

Much attention has been given to a peculiar mutant-enzyme of DNA polymerase I, isolated from the polA¹⁰⁷ mutant of *E. coli*. The mutant enzyme has normal polymerase activity and 3' - 5' exonucleolytic activity, but lacks the 5' - 3' exonucleolytic activity. The enzyme is able to synthesize a new strand with nicked double-stranded DNA as substrate, in the absence of concurrent DNA breakdown.

Project No. A 2.1

Title: Studies in vitro to elucidate the mechanism of repair processes

Research workers: Dr. R. A. Oosterbaan, Drs. A. Hout, Ir. B. van Dorp,

Drs. H. L. Heijneker, Dr. Ir. C. A. van Sluis, Dr. Ir. P. van de Putte, Dr. P. H. Pouwels,
Dr. W. F. Stevens

Progress report:

1) The important function of the ATP-dependent nuclease in recombination and repair in *E. coli* stimulated further research concerning the properties of a related enzyme in *Micrococcus luteus*. The mechanism of action of this enzyme has been investigated with partially purified or extensively purified enzymes (approximately 50 per cent pure).

Glycerol gradient sedimentation analysis as well as phase partition experiments (polyethylene glycol-dextran) have shown that the enzyme forms a very stable complex with linear double-stranded DNA from phage T7, but not with single-stranded DNA or circular double-stranded DNA. For this reaction ATP is not required; due to the instability of the enzyme in the absence of magnesium ions the binding experiment could only be performed in the presence of magnesium ions. Two enzyme molecules will bind as a maximum to a linear duplex, probably one to each end.

Double-stranded DNA with sticky ends from phage lambda still binds the enzyme but T7 DNA treated with exonuclease III to get more extended single-stranded ends, is completely incapable to bind the enzyme.

Free DNA molecules do not compete with molecules initially bound to the enzyme.

Results from kinetic experiments suggest that the formation of the enzyme-DNA complex is a necessary intermediate in the overall degradation process. Degradation after the formation of the DNA-enzyme complex is a first order process, its rate being proportional to the concentration of the complex. At 0° C one enzyme molecule needs about 30 min to degrade one T7-DNA molecule with a MW = 26×10^6 .

2) The investigation to show that the (hypothetical) mechanism through which UV-induced damage in DNA can be repaired by the successive actions in vitro of a number of enzymes, has been proceeded. Now it has become evident that the combined action of highly purified UV-specific endonuclease, polymerase I and polynucleotide ligase cannot lead to the repair of UV-damaged DNA. For the

action of polymerase I the hydrolysis of the phosphate ester bond near the thymine-dimers, by the endonuclease must be followed by the introduction of another break by means of exonuclease III to get the appropriate terminal group. Only under these conditions the breaks which result from the action of UV-specific endonuclease on UV irradiated RF-DNA from phage Φ X174 can be restored completely .

3) Much attention has been devoted to the characterization of the biochemical lesion of the *E. coli* mutant KMBL 1364 exr^- (now *polA* 107). Phenotypically this mutant exhibits a pronounced sensitivity towards X-rays and is incapable to restore MMS-treated phages.

Biochemical analysis has revealed that this mutant synthesizes an abnormal polymerase I. The following characteristics of the enzyme have been established:

- the enzyme behaves similarly to the wild type enzyme on SDS polyacrylamide-gel electrophoresis,
- mutant and wild type enzymes possess comparable 3' \rightarrow 5' exonucleolytic activities and polymerase activities (exonuclease III treated DNA),
- the mutant enzyme is devoid of any 5' \rightarrow 3' exonucleolytic activity, contrary to the wild type enzyme

4) Extracts of *E. coli* K12 show a nuclease activity which is absent in the isogenic recB and C strains. The nuclease activity is not stimulated by Mg^{++} or ATP and is even inhibited at higher concentrations of Mg^{++} than $10^{-2}M$. Extracts of RecA strains show a much higher activity than of the wild type strain. Studies are in progress to isolate the enzyme.

Project No. A 2.2 and A 2.3

Title: Identification of gene products determining radiation-sensitivity of bacteria.

Research workers: Dr. Ir. P. van de Putte, Drs. A. Hout, Dr. F. Palitti, B.W. Glickman, M.Sc., Dr. Ir. C.A. van Sluis

Progress report:

It has been shown that the ATP-dependent exonuclease was absent in RecB C cells and was more active in extracts of RecA cells than in Rec⁺ cells.

First it was thought that the higher activity of the enzyme in RecA cells was due to the absence of the RecA gene product that would inhibit the ATP exonuclease. It was found, however, that the difference between Rec⁺ and RecA extracts was due to different amounts of inhibitor DNA in those extracts. The RecA DNA, purified from the extracts, bands in two peaks in a neutral sucrose gradient, whereas the Rec⁺ DNA bands in one peak. Only the DNA from the RecA extracts, which corresponds with the DNA of Rec⁺ extracts, inhibits (by substrate dilution) the ATP-exonuclease activity.

The difference between the two DNA's is not found when the DNA is isolated from cells which are lysed by SDS. Apparently the DNA in the RecA cell extracts becomes more fragmented than in Rec⁺ cell extracts and it was found that this fragmentation is due to an ATP-independent nuclease. This nuclease, which activity is much lower in Rec⁺ cell extracts and unmeasurable in RecB C extracts, works only with very low Mg²⁺ concentration and the activity is lost by RNase treatment. A strong stimulation is found when tRNA is added to the extract. The enzyme will now further be characterized and its relation to the RecB C and RecA genes established.

DNA-free extracts of the RorA mutant contain more ATP-nuclease per mg of protein than extracts of the wild type. The ATP nuclease from Ror⁺ and Ror⁻ cells are purified now to such an extent that comparative experiments can be started with the two enzyme preparations in the near future.

As concerned the identification of the RecA gene product, Hfr's were isolated which have their point of origin very close to the RecA gene. These Hfr's were isolated using the phenomenon that F-factors can become integrated at any site of the bacterial chromosome with the help of bacteriophage Mu. From these Hfr's it will be tried to isolate RecA containing episomes as a first step for the isolation of RecA carrying phages which will be very useful for the isolation of the RecA gene products.

In 1972 no work was done on the identification of uvr gene products.

Project No. A 2.4

Title: The isolation of well defined multiply marked mutants of Escherichia coli K12

Research workers: Prof. Dr. A. Rörsch, Dr. P. van de Putte, Dr. I. E. Mattern, B. W. Glickman, M. Sc.

Progress report:

Mutants of the original E. coli K12 F⁺ strain were isolated by introducing the desired genetic markers via transduction. Thus the strains are never brought into direct contact with mutagens and the genetic quality of the strains can be ensured. In this way several hundred strains containing auxotrophic markers have been isolated. Furthermore, into a number of these strains, various mutations have been transferred which affect radiation sensitivity. With the exception of the small section of chromosomal DNA which is introduced by the transduction, the so isolated radiation-resistant strains form strict isogenic partners.

A master strain was produced, KMBL 1835 lacZ 001, malB78 serA101 metA79 metE72 proC65 trpS1 pheA001, his-51 bio-87 thyA301, towards which can be transferred any of the genes determining radiation sensitivity of the series uvrA, B, C, D, E, F, polA, recA, B, C, rorA, exrA, lonA by transduction, by selection for the + allele of the neighbouring auxotrophic marker.

Laboratory of Molecular Genetics, State University Leiden, The Netherlands

(in cooperation with the Medical Biological Laboratory TNO, Rijswijk 2100)

Title: IDENTIFICATION OF THE PRIMARY LESIONS INDUCED BY
IONIZING RADIATION

Summary:

Ionizing radiation induced single-strand breaks, double-strand breaks and nucleotide damage, i.e. damage not accompanied by a break, in the most important cellular target DNA. It has been shown that in phage DNA nucleotide damage may be responsible for more than 80 per cent of the lethality. Not all nucleotide damage is lethal, however.

Part of the damage induced by γ -rays in phage DNA can be repaired by the excision repair system of the host bacterium. Repair of damage inflicted under oxygen, however, does not need the "UV-endonuclease".

A small fraction of the radiation damage causes mutations in bacteriophage, both in single- and in double-stranded DNA phages. Often mutation induction is higher under oxygen than under nitrogen. Replication of the DNA is not necessary for expression of pre-mutational damage.

Sensitization of cells to ionizing radiation by the presence of the well-known sensitizers paranitroacetophenone and triacetoneamine-N-oxyl, was found not to be due to reaction of these compounds with DNA radicals, suggesting that the so-called "direct action model" of sensitization does not apply. The presence of these compounds during irradiation does have an influence on the repairability of the radiation damage in DNA.

Project No. A 3.1

Title: Induction of mutations in bacterial and mammalian cells and viruses and
in biologically active DNA by physical and chemical agents

Research workers: Ir. J. F. Bleichrodt, Drs. J. Vreeswijk, Prof. Dr. A. Rösch,
Dr. R. A. Oosterbaan

Progress report:

An ochre mutant of bacteriophage Φ X174 contains the codon TAA in its DNA, an amber mutant, the codon TAG, and an opal the codon TGA. In the previous annual report it was reported that the conversion of a particular ochre mutant into an amber by γ -irradiation showed an oxygen effect. This was found not to be a general phenomenon.

Attempts to convert an opal mutant into an ochre, representing the transition of guanine into adenine, have failed thus far. The opal mutants available also could not be mutated into ochres by chemical means, indicating that the amino acid inserted by the ochre suppressor in the bacterium used, yielded non-functional proteins and not that the conversion $G \rightarrow A$ by irradiation cannot occur.

Amber mutants in cistron A of bacteriophage Φ X174 can be reverted by γ -rays to pseudo-wild types, i. e. to particles able to grow in a suppressorless host. Since these ambers do not show any replication of the double-stranded form of Φ X174 in the non-permissive host, it can be concluded that replication of the double-stranded DNA is not necessary for expression of the mutation induced.

Induction of mutations by γ -rays was also studied in the double-stranded DNA phage T7. Amber mutants of T7 irradiated in nutrient broth are mutated to pseudo-wild type particles. The efficiency of induction varies between 10^{-6} to 8×10^{-6} mutants per lethal hit per phage particle for amber mutants in different cistrons irradiated under oxygen. Under nitrogen the efficiency is lower for all mutants tested.

Induction of revertants in mutants in cistron 1 and 5, which code for T7 RNA polymerase and T7 DNA polymerase respectively, shows that DNA replication is not necessary for expression of radiation-induced mutations. Since the first few cistrons of T7 are transcribed by the bacterial RNA polymerase, and cistron 5 by the T7 RNA polymerase, it may be concluded that these polymerases can "read" the pre-mutational lesions induced by the radiation, unless these lesions are

first repaired by host repair enzymes.

With the exception of the polA mutation in Escherichia coli, which results in a lower induction of mutations in T7, induction was the same on various E. coli strains deficient in their repair systems.

An increase in induction of mutations, which is obtained in the presence of oxygen, is not obtained by irradiation in the presence of paranitroacetophenone or triacetoneamine-N-oxyl. With respect to mutation induction in phage these compounds, which like oxygen sensitize cells to ionizing radiation, therefore show a behaviour different from oxygen.

Project No. A 3.2 and A 3.3

Title: Chemical and physical identification of lesions in nucleic acids

Research workers: Dr. J. de Jong, Dr. G. P. van der Schans, Drs. J. J. van Hemmen,
Ir. J. F. Bleichrodt

Progress report:

1) When the double-stranded circular DNA of the bacteriophage PM2 is irradiated in buffer in the presence of oxygen, about 5 per cent of the lethality is due to double-strand breaks, assuming that the efficiency of inactivation of the DNA by a double-strand break is 100 per cent. About 85 per cent of the lethality is due to nucleotide damage, i.e. damage not resulting in strand breakage. Not every nucleotide damage is lethal, however. Of the nucleotide damage which is lethal in the biologically active single-stranded circular DNA of PM2, which can be obtained from the double-stranded DNA, only 30 per cent is lethal if present in double-stranded DNA.

Radiation-induced single-strand breaks contribute for about 10 per cent to lethality in PM2 DNA. Only about 2 per cent of all single-strand breaks is lethal. These data confirm the less accurate results previously obtained with the circular double-stranded replicative form of phage Φ X174.

Investigations on the lethal damage in DNA inflicted by irradiation of phage PM2 in protective media were less successful. The phage coat is very sensitive to radiation and DNA cannot be recovered quantitatively after irradiation. Therefore the direct effect of radiation on PM2 DNA is now being investigated by irradiating the DNA in nutrient broth at -196° . The presence of nutrient broth in the medium excludes the application of the previously mentioned filter

technique to separate the various DNA components in irradiated samples. This separation is obtained now by an improved method of sucrose gradient sedimentation.

2) When DNA of the bacteriophage T7, heavily labeled with ^3H in the adenine bases at C-8, is irradiated with γ -rays adenine is liberated from the DNA and also water is formed. When irradiated DNA was degraded by pancreas DNAase I and with snake venom phosphodiesterase, mixed with irradiated 5'-dAMP and the radiation products separated, the products of 5'-dAMP and those of DNA containing ^3H appeared to be the same. Therefore identification of the products is performed using irradiated 5'-dAMP. Besides free adenine, 4,6-diamino-5-formamidopyrimidine was detected, together with a third main product which disintegrates during purification. A few of the disintegration products have been characterized by mass spectrometry.

3) Radiation damage in DNA of bacteriophage T1 sustained by irradiation of the phage in protective media under nitrogen, can be repaired by the excision repair system in E. coli, because the phage was found to show a greater sensitivity on a Uvr^- than on a Uvr^+ strain. The survival of T1 did not differ on these strains if irradiation was performed under oxygen. On a Pol^+ and a Pol^- strain there was a difference in sensitivity both after irradiation under oxygen and after irradiation under nitrogen. These observations suggest that the "UV-specific" endonuclease recognizes only γ -ray damage inflicted under anaerobic conditions. This conclusion was supported by experiments with the bacteriophages T4 and T4v. This phage codes for its own "UV-specific" endonuclease, and the latter strain carries a mutation in the endonuclease cistron.

4) In the previous Euratom report, it was shown that the so-called "direct action model" of sensitization of DNA by electron affinic sensitizers does not apply to irradiation of dry DNA in the presence of the sensitizer paranitroacetophenone (PNAP). This was also shown to be the case for triacetoneamine-N-oxyl (TAN). Also in the rapid-mixing apparatus these compounds were found not to sensitize the inactivation of biologically active DNA if the irradiated DNA was mixed with one of the sensitizers within 1.5 ms after irradiation. In steady state experiments where DNA is irradiated in buffer in the presence of TAN, the sensitizer does react with DNA, since the survival of the DNA was found to be lower on spheroplasts of a Uvr^- than on those of

a Uvr^+ strain. DNA irradiated in the presence of PNAP does not show a difference in survival on these strains.

When bacteriophage T1 is irradiated under anoxic conditions in the presence of PNAP the difference in survival of the phage on a Uvr^+ and a Uvr^- strain is decreased which is an effect similar to that of oxygen. TAN on the other hand causes an increase in the difference in survival.

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Department of Radiation Genetics and Chemical Mutagenesis

Contract No. 102-72- 1 BIAN

Prof. Dr. F.H. Sobels

The Effects of Radiation on Genetic and Biochemical Systems

Research is directed to studying the induction of mutations and chromosome aberrations in somatic and germinal tissues of several mammalian species, including man.

A system has been developed for the selection of 8-azaguanine resistant mutants in diploid human cell strains; it was found that 8-azaguanine is not only selecting, but also inducing mutants. Mutants are being tested for residual enzyme activity and their ability to grow on restrictive HAT-medium.

Groups of mice have been given exposures ranging from 100 R to 600 R of X-irradiation. Chromosome aberration frequencies in bone marrow and testes are being determined at various time intervals after exposure. A first somatic-germ cell correlation has been obtained for one dose and time interval.

Testes biopsies have been tried as a source of material for studies on the radiosensitivity of human male germ cells. Cultured meiotic cells did not, however, develop further than late pachytene.

Attempts to induce cell transformation are being continued with human diploid skin fibroblasts.

Drosophila is an ideal organism for studies on the induction and recovery of radiation induced changes at the chromosomal level. The sophisticated genetic systems which are available in this organism permit an analysis and manipulation of events which can only be observed cytologically in other systems. The models derived from Drosophila are essential for the interpretation of data used to estimate the genetic hazards to man. The results of the past year's research are summarized below.

Treatment of females with caffeine affects the recovery of dominant lethals and chromosome loss induced by the irradiation of spermatozoa in males. Maternal genotype was also found to be a significant factor.

Heterozygosity for structural rearrangements of the two major autosomes did not affect the induction and recovery of translocations when stage-7 or stage-14 oocytes were irradiated.

By using dose fractionation, it has now been convincingly demonstrated that many chromosome breaks induced in spermatids remain open until after fertilization.

Autosomal non-disjunction has been studied by using compound autosomes. The evidence obtained so far indicates that there is no threshold for the induction of non-disjunction in stage-7 or-14 oocytes. There was no apparent dose rate effect on non-disjunction induction in stage-7 oocytes.

The relative breakability of euchromatin and heterochromatin and the absolute breakability of the latter have long been subjects of discussion. Work reported here indicates that the breakability of heterochromatin is not in accordance with currently accepted hypotheses.

By repeating techniques used in mouse genetics, with Drosophila, it is possible to check the validity of the genome mutation rates in the mouse.

Project No.: B 1.1
Participant(s): D. Mendelson
Title: Effects of Changing the Maternal Environment with
with Chemical Inhibitors on the Repair of Chromosome
Breaks Induced in Male and Female Germ Cells

Caffeine is consumed in large quantities throughout the world. Tests for mutagenicity have given a different response in different test systems, and caffeine by itself does not seem to constitute a serious risk as an environmental mutagen. In microorganisms caffeine acts as an inhibitor of dark repair, and thus potentiates radiation damage. The effect of caffeine on the repair of chromosome breaks in *Drosophila* was studied in several different ways:

- 1) When males were fed for two days on 0.15% caffeine in 10% honey and then exposed to 1000 R, a non-significant increase of the II-III translocation frequency over the controls was observed in sperm, spermatids and spermatocytes.
- 2) When females of Nöthel's (Mutations Res. 10: 463, 1970) R \ddot{O} I stock (which exhibits radioresistance in stage-7 oocytes) were injected with 0.13% caffeine, and then exposed to 3000 R X-rays, a significant increase of 7-16% dominant lethality was observed in comparison to that in the controls. Caffeine treatment increased the X-ray response of the radioresistant stock to the level of the normal (+⁶⁰) non-radioresistant stock. In the latter stock, the effect of caffeine was far less pronounced and not significant. This observation suggests, that radioresistance in the R \ddot{O} I stock is brought about by a biochemical system that can be inhibited by caffeine.
- 3) Young females of different stocks were injected (0.15-0.17%) or fed (0.14-0.2%) with caffeine and mated two days after eclosion to males, which had been irradiated with 2000 R. A significant increase was observed then for the dominant lethal frequency in the paternal genome. If $y\ sn^3$ females were treated with caffeine, loss of the paternal X-chromosome was similarly increased. However, the latter effect was less pronounced when "Oster" females ($y\ sc^{s1}In49\ sc^8; bw; st\ p^p$) were used. Very high frequencies of paternal sex-chromosome loss were obtained when irradiated males were mated to Ubx females. Caffeine treatment of these females

did not affect significantly the frequencies of either chromosome loss or intra-chromosomal rearrangement. This indicates that the Ubx females probably have a very inefficient repair system.

These results provide evidence that the repair system in *Drosophila* females (Proust, Compt. Rend. 269: 86, 1969, and Proust, Sankaranarayanan and Sobels, Mutation Res. 16: 65, 1972, and Würgler and Maier, Mutation Res. 15: 61, 1972), responsible for repair of restitution of breaks in both the maternal and paternal genomes, can be effectively inhibited by caffeine. In accordance with this hypothesis, when females were treated with caffeine, it was found that fewer translocations were recovered from irradiated male gametes.

Project No.: B 1.3
Participant(s): Dr. K. Sankaranarayanan
Title: Effects of Structural Heterozygosity on the
Induction of Autosomal Translocations in
Female Germ Cell Stages

The induction of translocations between chromosomes II and III has been studied in oocyte stages 14 and 7 from females that are structurally heterozygous for these chromosomes ($\frac{SM5}{+}; \frac{TM3\ Sb\ Ser}{+}$). The general rationale of this experiment is the following: exchanges between chromosomes in stage-7 oocytes depend on pairing relationships, whereas in stage-14 oocytes on chance associations; if homologous chromosomes can be effectively inhibited from pairing for exchange, the chromosomes so inhibited undergo distributive pairing, one which is dictated by chromosome length and not by homology. In a structural heterozygote, such as the one used (the SM5 and TM3 chromosomes are believed to inhibit exchange pairing), there is thus a greater opportunity for heterologous pairing. If translocations can be induced between the autosomes and provided there are no complications in segregation following their induction, such translocations should be recoverable at frequencies higher than under conditions when normal females are irradiated.

The results thus far available show (i) in stage-14 oocytes irradiated with 500 R, out of 1112 gametes tested, 9 translocations were recovered (0.8%); none of these translocations could be kept for more than a generation or two following their recovery (ii) in stage-7 oocytes, irradiated with 3000 R, the rate of recovery is quite low... 1 in 523 gametes tested (0.2%). The frequency in stage-14 oocytes appears higher than the one recorded by Traut in normal females (0.36% at 500 R); however, it appears likely that the higher rate in the present study is probably due to improved sampling technique (8 hrs of egg-laying versus 24 hrs used by Traut). The rate for stage 7 ... 0.2% is not inconsistent with that obtained by Traut (0.32% at 3000 R).

The results thus demonstrate that structural heterozygosity does not have any appreciable effect on the rate of recovery of autosomal translocations from either of the germ cell stages examined in this respect. It is proposed to terminate this project as soon as experiments currently underway are completed.

Project No.: B 1.5
Participant(s): Prof. Dr. F.H. Sobels
Title: How long do breaks induced in pupal spermatids remain available for interaction?

A number of further dose-fractionation experiments were carried out to determine at what stage of spermatogenesis (in *Drosophila* pupae) X-ray induced breaks (or potential breaks) remain available for interaction with breaks induced in the same cells, when mature spermatozoa. A first radiation exposure of 500 R was given to spermatids in 24-hour or 0-hour pupae. The second fraction of 1000 R to the same cells, as mature spermatozoa, in inseminated females. The yield in the fractionated group was compared with that expected on the basis of additivity of interaction between breaks induced by the single fractions (obtained from simultaneous parallel experiments). Previous observations had shown that following irradiation of spermatids in 48-hour pupae, the translocation frequency in the fractionated group was significantly higher than the sum of the yields of the separate fractions. This result corresponds to our findings for adults, but stands in contrast to earlier observations of Oster (1955) and Falk (1961). After irradiation of 24-hour pupae, additivity was observed in three out of four experiments, and complete interaction in a fourth. Following irradiation of 0-hour pupae, no interaction was found. The transition from interaction to additivity thus occurs in spermatids of pupae around 24 hours after puparium formation. The finding that lesions in spermatids remain available for interaction with damage which is induced 4-5 days later after insemination of the female is of interest. The frequencies of recessive lethals and translocations can be modified by contrasting post-treatments, following irradiation of spermatids under anoxia (Sobels 1965; Watson 1967). In view of the recent observation by Proust, Sankaranarayanan and Sobels (1972), and Mendelson, Clark and Sobels (1972), that the expression of genetic damage induced in sperm cells can be profoundly influenced by both the genetic and physiological conditions of the oocyte, it seems worthwhile to investigate whether post-treatments of spermatids makes them more or less susceptible to undergo repair proceeding fertilization in the female.

Project No.: B 2
Participant(s): Prof. Dr. F.H. Sobels
Title: Radiation induction of non-disjunction of autosomes

Non-disjunction is an obvious hazard to man, because recent surveys (Jacobs, Proc. 4th Int. Congr. Human Genetics, Paris, September 1971) show that 30-40% of spontaneous abortions and 0.4-0.8% of live-born children are associated with aneuploidy, and hence presumably arise from non-disjunction. Until now non-disjunction could only be measured for the sex-chromosomes or the small fourth autosome, because trisomy or monosomy for one of the large autosomes is lethal in *Drosophila melanogaster*. For non-disjunction of the X-chromosomes an unusual dose-effect relationship with a threshold up to 1000 R has recently been reported by Traut (Mutation Research 10: 125, 1970). To investigate whether Traut's observation is of more general applicability, a method was devised to study non-disjunction of the large autosomes by employing isochromosome stocks. Advantage is taken of the fact that in the female, isochromosomes usually disjoin regularly, whereas in the males they show disjunctive and non-disjunctive segregation with equal frequencies. This system makes the quantitative recovery of non-disjunctive progeny possible, because disomic and nullosomic female gametes will result in viable zygotes after fertilization with nullosomic or disomic sperm, respectively.

The induction of non-disjunction by X-irradiation of the second chromosome in stage-7 oocytes has been studied by employing various isochromosome stocks. Determination of egg-hatchability has been used to correct for varying degrees of segregation in males carrying different isochromosomes. Even at exposures as low as 250 R the frequency of non-disjunction is significantly higher than in the controls. No evidence has been obtained for the existence of a threshold. In the stage-7 oocytes, the induction of non-disjunction increased linearly with radiation exposure over a range of 250-3000 R and thus seems to reflect a single-hit event. These findings could be of significance for the evaluation of genetic radiation hazards in man. In slightly younger oocyte stages the induction of disomic eggs followed dose-square kinetics. The frequency of nullosomic eggs rises exponentially with radiation exposure, presumably as a consequence of increasing chromosome loss, resulting from unrestituted breaks in each of the two maternal isochromosomes. Furthermore, it was observed that the late stage-7 oocytes were more sensitive to the induction of non-disjunction than earlier stages.

Project No.: B 2
 Participant(s): Prof. Dr. F.H. Sobels
 Title: The induction of non-disjunction of compound second chromosomes in stage-14 oocytes of X-rays

The findings recorded for the induction by X-rays in stage-14 oocytes are controversial. After exposure to 500 R, Day and Grell (Mutation Research 3: 503, 1966) obtained values similar to those expected from stage-7 oocytes, provided linear extrapolation from 4000 R data for stage-7 oocytes is correct. For both the X and the fourth chromosomes, Kiriazis and Abrahamson (Genetics 60: 193, 1968) did not observe an increase over the control values. The latter result was confirmed by Traut (Mutation Research 10: 156, 1970), who categorically concludes that a dose of 400 R does not induce non-disjunction in either mature or immature (Traut, Mutation Research 10: 125, 1970) oocytes. More recently, however, Clark and Sobels (Mutation Research: in press) and Sobels and Clark (Abstr. 3rd Europ. Dros. Res. Conf., Milan, Sept. 1972, ed. Barigozzi), by making use of compound second chromosomes, were able to show that, in contrast to Traut's findings, exposures of 500, and even 250 R do significantly raise the non-disjunction frequency in stage-7 oocytes over that in the controls. A re-investigation of the induction of non-disjunction in stage 14-oocytes, using this method, seemed therefore indicated.

Four-day old females of the genetic constitution C(2L)RM, j⁶³; C(2R)RM, px were exposed to 400 R and mated to C(2L)RM, bpr; C(2R)RM, vg males. To record stage-dependent sensitivity differences, egg samples were collected from 9.00 a.m. until 5.00 p.m., and from 5.00 p.m. until 9.00 a.m., over a period of 72 hours in total; in this context only the data for the first two broods, sampled during 24 hours after exposure to irradiation, are presented (see table).

Pooled data of 7 replicate experiments

Brood	Hours after irradiation	No. progeny	Disomic eggs No.	Disomic eggs %	Nullosoomic eggs No.	Nullosoomic eggs %
A	0 - 8	682	6	0.88	11	1.6
B	8 -24	2,734	26	0.95	24	0.88
Controls		12,142	50	0.41	42	0.35
Stage-7 oocytes (500 R)		7,393	70	0.94	33	0.44

Considering first the data for disomics, it may be noted that their frequency does not significantly differ between broods A and B, and hence the data of these two sampling periods were pooled. The frequency of 0.93% thus obtained, is significantly higher (at the 0.01% level, Kastenbaum and Bowman, Mutation Research 9: 527, 1970) than in the controls, and does not at all differ significantly from that found after exposing stage-7 oocytes to 500 R. The present data thus confirm earlier findings that there is no difference in radiosensitivity with regard to the induction of non-disjunction between stage-14 and stage-7 oocyte stages. Moreover, they are in line with the findings of Day and Grell for X-chromosome non-disjunction in stage-14 and slightly earlier oocyte stages, but different from those recorded by Kiriazis and Abrahamson, and Traut.

With regard to nullosomics, it can be seen that these appear to be induced at higher frequency in brood A, than in brood B, though the difference is not significant. The frequency obtained in brood A is significantly higher ($P < 0.01$) than in the controls, and that induced by 500 R in stage-7 oocytes. A similar result has been recorded by Traut, and the different findings for disomics and nullosomics clearly originate from the fact that chromosome breakage phenomena contribute to the induction of nullosomics, but not to that of disomics.

Project No.: B 2
Participant(s): B. Leigh
Title: Effect of dose rate or autosome non-disjunction

Recently, Traut (Mutation Research 12: 321-327, 1971) has claimed that when Drosophila melanogaster oocytes are irradiated the amount of induced non-disjunction is dependent on the way in which the radiation is given. Namely, a dose of 1,800 R given at 850 R/min. produced the same amount of non-disjunction of the sex chromosomes when the dose was given all at once and also when it was given in two fractions separated by a 1-, 3-, or 5-hour interval. However, a much lower frequency of non-disjunction was induced when the same dose was given chronically at a rate of 10 R/min. Several experiments have now been carried out to investigate whether there is also a dose rate effect on the induction of non-disjunction of isochromosomes. Two dose rates are being used, 2,600 R/min. and 10 R/min. The exposure levels which have been selected are 1,000 R and 1,500 R. The first eight experiments have not provided evidence of a dose rate effect for the induction of non-disjunction of compound autosomes, when immature oocytes are irradiated.

Project No.: B 3
Participant(s): A. Schalet
Title: Quantitative and qualitative characterization of
radiation-induced breakpoints in the proximal
heterochromatin and adjacent euchromatic regions
of the X-chromosome

Three of the elementary questions which can be raised with respect to the distribution of radiation induced chromosomal breaks in *Drosophila* are 1) What is the significance of the distribution of breaks in euchromatic segments?, 2) Are breaks distributed within heterochromatic segments at random?, 3) What is the apportionment of breaks between eu- and heterochromatic segments?, i.e., is there a difference in the relative effective breakage (breakage and rejoining) between eu- and heterochromatic segments? The analysis of X chromosome breakpoints provides the best approach to these questions. It is fairly clear that the distribution of breaks in euchromatic segments correlates well with measurements of DNA content along the length of the polytene chromosome. Answers to the remaining questions are less satisfactory. Work done under this project was intended to help to clarify the situation. The work of Rudkin (1969) and Gall et al. (1971) indicate that heterochromatic segments do not replicate during polytenization. As a consequence of this fact and our own cytogenetic analysis of the proximal heterochromatic-euchromatic segment of the X (Schalet and Lefevre, submitted for publication), it can be seen that previous calculations of the proportion of total chromosome breaks assigned to the heterochromatic segment must have been overestimated to some extent. The degree of overestimation is uncertain but, until it can be worked out, the relative effective breakage of euchromatin vs. heterochromatin remains uncertain. Experiments designed to determine the relationship between breakage and the size of the heterochromatic segment present in the X chromosome have produced results which appeared to rule out a simple quantitative relationship. Thus Muller (1944), confirmed by Wagoner (1968), reported that equal frequencies of X-ray-induced X-autosome translocations were recovered from two X-chromosomes which differed in their mitotic lengths by a factor of two because of a seven-fold difference in the amount of X-chromosome heterochromatin. Muller (1944) also reported equal frequencies of X-ray-induced deficiencies at the tip of the X for two chromosomes in which the amount of heterochromatin, just proximal to the marker used to detect deficiencies, differed by a factor of four. No data were reported for Muller's experiments, but the data for Wagoner's translocation experiments are conclusive.

Since experiments of the second type mentioned in the previous paragraph provide a direct estimate of breakage in X chromosome heterochromatic segments of different sizes, we have repeated this type of experiment. Preliminary results are in disagreement with Muller's observation. The experiments were designed to select for deficiencies with a breakpoint between two specific markers which delimited a heterochromatic segment. The loss of either marker, but not both, was compared in two chromosomes which differed by a factor of about four in the size of the heterochromatic segment between the markers. Following the irradiation of adult males with 3,000 R and sampling of mature and near mature sperm, the relative frequency of loss for the left marker, ac^+ , was 3:1 and for the right marker, $su(f)^+$, was 6:1. The difference between the two chromosomes for the loss of each marker was statistically significant, and the ratio for the combined losses was approximately proportional to the relative sizes of the heterochromatic segments in each chromosome. These data are considered preliminary because the detailed genetic analysis of the heterochromatic and euchromatic breakpoints was limited due to the fact that the majority of the flies carrying the $su(f)^-$ chromosome failed to breed and there was an unusually high control value for the ac^+ losses in one of the chromosomes. The latter event probably represents a high incidence of X-Y exchanges rather than an inherently high spontaneous mutability in the stock. If these results are reproducible it would mean that breakage in the X chromosome heterochromatic segment is indeed proportional to the size of the segment. Furthermore, we would infer that the apparent lack of proportionality in the X autosome translocation experiments of Muller and Wagoner might well lie in the uncertainty of the relative breakability of euchromatic segment vs. heterochromatic segments or secondary factors which influence the recovery of interchromosomal rearrangements.

Project No.: B 5
Participant(s): Dr. K. Sankaranarayanan
Title: How valid are the genome mutation rate estimates in
the mouse? A reconstruction experiment with
Drosophila.

An attempt is being made to examine the efficiency of the inbreeding technique used by mouse workers to screen for the presence of autosomal recessive lethals. Two approaches are employed, namely, computer simulation and model experimentation with Drosophila. The basic principle is that (i) when there is brother sister mating between two heterozygotes carrying an autosomal recessive lethal, survival will be reduced by 25% over that in controls (ii) when there is mating between a father (which is heterozygous for the lethal) and daughter (50% of which are expected to carry the lethal) survival will be reduced by 12½% over that in controls. To be more precise, the survival values will have a distribution around mean survivals mentioned above and the question asked is how precisely one can assign a given survival to a distribution belonging to crosses of types a) controls - none of the parents carry the lethal, b), both parents carry the same lethal, c), father carries the lethal but the daughter may (50%) or may not (50%) carry the lethal.

The computer simulation shows that (i) when samples of 100 implants are repeatedly drawn from an imaginary population and distributions of survival plotted when the control mean survival is 92% (the level actually observed in mouse studies) and when the expected mean survival in the "lethal" group is 69% (75% of 92%), these distributions do not overlap; if instead the expected mean survival in the lethal group is 80.5% (87.5% of 92%) and the control survival as before, the distributions overlap slightly such that the efficiency of identifying a lethal cross is still relatively high and is not inconsistent with what Lünig has claimed (60-75% efficiency of detection) with somewhat smaller sample sizes.

The Drosophila experiments, the material for which was derived from a radiation study (lethals and non-lethals for the second chromosome) show that the mean survival is 71.5% in the controls, 55.3% in the situation where the expected mortality is 25% over the controls, and 64.3% in that where the expected mortality is 12½% over the controls. The "lethal" distributions show a considerable overlap with that of the control; it appears that the overlap is indeed expected under the above conditions

where the mean survivals are lower than in the mouse. This means that the *Drosophila* approach may not be quite comparable to that obtained in the mouse. These results, still not extensive, suggest that at face value, Lüning's technique is probably adequate. The experiments are continuing.

Project No.: B 6.1
Participant(s): Dr. J.W.I.M. Simons, Drs. M.C.E. van Diggelen,
Ir. A.A. van Zeeland
Title: Mutation in Diploid Somatic Cells in Vitro

A system for the selection of 8-azaguanine resistant mutants from diploid human cell strains has been developed. The selection of mutant cells appeared largely influenced by a phenomenon, known as metabolic cooperation, which turns mutant cells into phenotypically wild-type cells, when they are in contact with wild-type cells. As a consequence of metabolic cooperation, mutant cells can only be selected at a low cell density.

At first mutants were selected from inocula of 10^4 cells per 100 mm Petridish, which were stimulated to grow by the addition of $3,5 \times 10^5$ mutant feeder cells. This gave rise to a great variation in mutant frequencies. The selection system became reproducible by the use of a richer medium which made the feeder cells superfluous and by the use of selected sera, which do not interfere with the effect of 8-azaguanine (see DeMars and Held, Human Genetik 16, 87-110, 1972). For the exact determination of the mutation frequency a number of technical problems still remain to be solved. Thus, for example, the expression time of the mutations and the correction for metabolic cooperation and cloning efficiency should be known.

The influence of 8-azaguanine concentration on mutant frequency and residual enzyme activities of mutants has been subjected to extensive study. It was observed that almost all mutants selected at the low concentration of $1 \mu\text{g/ml}$ 8-azaguanine exhibit residual enzyme activity, while this is not so for mutants selected at the high concentration of $5 \mu\text{g/ml}$ 8-azaguanine; among these practically no enzyme activity was found. In view of these qualitative differences between mutants selected at the two different concentration, the prediction was made that likewise different mutant frequencies were to be expected. It is of interest to note that this is not the case. These data are therefore not in agreement with the notion that 8-azaguanine acts only as a selective agent. Further research is aimed at elucidating this important question.

Project No.: B 6.2
Participant(s): Dr. J.W.I.M. Simons, Drs. M.C.E. van Diggelen,
Ir. A.A. van Zeeland
Title: Analysis of 8-azaguanine Resistant Mutants

A large number of mutants, selected at 1.2 and 5 μ g 8-azaguanine per ml, were isolated.

The mutants were tested for HGPRT-activity in lysed cells and intact cells and for their growth in HAT-medium. Most of the 29 mutants obtained from 1.2 μ g 8-azaguanine per ml showed residual enzyme activities. The degree of residual enzyme activity correlates with capacity of growth in HAT-medium. Mutants with about 4 percent residual enzyme activity are already capable of growth in HAT, which means that removal of pre-existing mutants is not possible when selection is carried out at 1.2 μ g 8-azaguanine per ml. Since only 4 of the 25 mutant selected at 5 μ g per ml show growth in HAT, removal of pre-existing mutants should be possible under this condition of selection.

On the whole, residual enzyme activities determined in lysed cells correlate well with the residual enzyme activities determined in intact cells. However, a few exceptions occur in which a high enzyme activity in lysed cells goes together with a low enzyme activity in intact cells. This suggests the existence of mutations affecting the system concerned with the uptake of hypoxanthine, which would imply another type of 8-azaguanine resistance.

Most of the mutants were able to grow at a higher concentration of 8-azaguanine (15 μ g/ml). As expected, the mutants without enzyme activity grew better at this concentration than the mutants with residual enzyme activity.

Project No.: B 6.3
Participant(s): P. van Buul
Title: Correlation between Radiation-Induced Chromosomal
Damage in Somatic- and Germinal Tissues

In order to obtain dose-response curves of radiation-induced stable chromosome aberrations in germinal and somatic tissues, seven groups of 10 mice have been irradiated with 0 - 100 - 200 - 300 - 400 - 500 and 600 R respectively. Chromosomal preparations of bone marrow and testis were made three months later. The scoring of the chromosomal aberrations is in progress.

In another experiment the "somatic-germinal" correlation of chromosomal aberrations is followed with time. Six time intervals have already been selected; 60 - 100 - 150 - 200 - 250 and 300 days. For each time interval 5 mice were given an exposure of 400 R. Sixty days after irradiation the frequency of visible translocations in the testis was about one quarter of the frequency of translocations in the bone marrow (i.e. 5.4% versus 19.8%). The evaluation of this experiment shall be further continued in 1973.

In cooperation with Dr. M. Lyon of Harwell and the TNO Primate Centre in Rijswijk, a preliminary experiment has been initiated to obtain dose-response curves from bone marrow, peripheral blood and testis in rhesus monkeys (*Macaca mulatta*). The radiation doses were 100, 200 and 300 rad.

To obtain more information on the possible heterogeneity of the spermatogonial stemcell population of the mouse, a dose fractionation experiment was started in collaboration with Dr. A. Leonard of Mol. Fractionated doses were 300 + 300, 500 + 100 and 100 + 500 R with a 24 hr interval. Single doses were 100, 400 and 600 R. This experiment is still in progress.

Project No.: B 6.4
Participant(s): Dr. A.D. Tates
Title: An Investigation into the Induction of Genetic
Radiation Damage in Male Germ Cells of Man and
Monkeys

For the evaluation of the genetic risks from ionizing radiation in man, information on the genetic radiosensitivity of human male germ cells is essential. Obviously, such information cannot be obtained from in vivo studies with free living persons. To circumvent this difficulty the possibility was explored to culture testis cells in vitro. The material for the in vitro studies consisted of 38 biopsies from men attending fertility clinics and of whole testes from a transexual and a patient with prostate cancer. The available material was very heterogeneous with respect to germ cell differentiation and 6 biopsies had no germ cells at all. All material was cultured at 31-33^o C in a gas atmosphere of 95% air and 5% CO₂. Most cells were grown in Ham's F10 medium supplemented with 15% newborn calfserum. The medium permitted good growth of testis fibroblasts and on several occasions it activated the mobility of spermatozoa for a period of six days. Testis material was treated in the following ways:

- 1) as small fragments (1 mm³ or larger) at the bottom of Petri dishes with variable amounts of medium;
- 2) as fragments of similar size floating on small pieces of lenspaper;
- 3) as cell suspensions embedded in a coagulum of human fibrinogen and thrombin that was covered by liquid medium;
- 4) finally the contents of short lengths of seminiferous tubules were transferred to cover slips and then embedded in a coagulum of chicken embryo extract and cock serum. The coverslips were kept in Petri dishes with a thin layer of liquid F10 medium.

The merits of the above culture regimens have been tested by cytological analysis of the dividing germ cells (modification of Evans, air-drying technique). Testis fragments that were kept in culture for longer periods of time (up to 5 weeks) have also been examined histologically. As a result of the heterogeneity and scarcity of suitable testis material it was impossible to make quantitative comparisons be-

tween results obtained under the above culture conditions. Generally speaking the number of dividing spermatocytes decreased with increasing culture times and after the 8th day in culture these cells were completely absent.

Histological analysis of cultured testis fragments showed that practically all pre-meiotic cells degenerate when reaching the late-pachytene stage. Such a "pachytene block" during in vitro differentiation of germ cells has also been described by other investigators. Evidently, under the present culture conditions, it is impossible to detect chromosome aberrations in dividing spermatocytes derived from irradiated spermatogonia and spermatocytes which were younger than late pachytene at the time of exposure. As a follow up of the above experiments it seemed worthwhile to estimate the genetic radiosensitivity of human spermatocytes which had passed the late-pachytene stage at the time of onset of the in vitro cultures. The most suitable cells for this purpose are spermatocytes during diakinesis-metaphase I. In vivo experiments with *Macaca mulatta* (Arsen'eva and Bochkov, 1963) and mice (Oakberg and diMinno, 1960; Chandley, 1972) have shown that such cells are very sensitive to aberration induction. Before using human material, we recently performed an in vitro experiment with a healthy testis of the monkey *Macaca mulatta*. Half of the testis was divided into fragments of 1 - 3 mm in diameter, which were then transferred to Petri dishes with F10 medium. The other half of the testis was mechanically transformed into a thick cell suspension in the same medium. Fifty percent of the fragments and the suspension was irradiated with 100 R and the rest of the material served as a control. Fragments and suspensions were fixed at intervals of 1, 3, 7, 10 and 22 hrs after irradiation. A preliminary analysis of the material has not yet yielded positive indications for the presence of chromosome aberrations in irradiated spermatocytes.

Project No.: B 7
Participant(s): Dr. J.W.I.M. Simons
Title: Induction of Cell-Transformation in Vitro by
X-rays

A. Test on induction of loss of contact inhibition in clones of diploid human skin fibroblasts after irradiation.

For this purpose cells were irradiated with 800 R and seeded at a density of 25,000 or 50,000 per Petridish. Under these conditions 5 to 25 clones are obtained per Petridish and metabolic cooperation, which could possibly interfere with cell-transformation is largely prevented. Only a minority of these clones become large enough to evaluate their morphology, as many of the clones show phenomena of cellular aging. The morphology of the fixed clones is evaluated in three categories: normal, transformed and partly transformed, the last category referring to some loss of contact inhibition throughout the clone. About 400 clones were classified from both control and irradiated cells. Following irradiation, one transformed clone and an increase in the number of partly transformed clones was found. However, upon isolation, 13 partly transformed clones failed to give rise to continuous lines. Thus induction of cell transformation has not yet been observed with certainty.

B. Test for the induction of continuous growth by irradiation of stationary senescent cells.

Irradiation of stationary senescent cells provides a selection system for transformed cells as the induction of continuous growth will lead to growth of stationary cultures. Moreover this system permits the detection of cell transformation when a long lag period is involved in the process of cell transformation. To this end 50 Petridishes per control and irradiated group (300 R) with 4×10^5 senescent cells each were initiated. The experiment is still in progress and so far no cell transformation has been observed.

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Laboratory for Physiological Chemistry
Contract No. 102-72- 1 BIAN
Dr. A.J. van der Eb

Cell transformation by oncogenic DNA viruses.

General description of the research activities:

Oncogenic DNA viruses are able to induce tumors in certain animals and transform cells in vitro. Available evidence suggests that only part of the viral genome, perhaps not more than two genes, are involved in oncogenic transformation. Knowledge of the mechanism of action of these genes, and of the way they persist in transformed cells, is of importance for an understanding of the process of (virus-induced) carcinogenesis. To obtain information on the functional and structural properties of the viral genes, that are involved in transformation, the following research projects have been carried out.

1. Transforming activity of adenovirus DNA

A new assay for determining infectivity and transforming activity of purified adenovirus DNA has been developed. This technique is now being used to transform cells with fragments of viral DNA in an attempt to isolate those parts of the genome that are involved in transformation.

Parallel to this work, experiments have been carried out on the effect of UV irradiation on the oncogenic potential of adenoviruses (non-oncogenic adenoviruses seem to acquire oncogenic potential upon UV irradiation).

2. Replication of adenovirus DNA

To investigate the functional properties of adenovirus DNA's in infected cells, a study of the mechanism of virus DNA replication in permissive cells was also undertaken.

3. Repair of radiation damage

In order to obtain a better understanding of the mechanism of radiation repair in mammalian cells, a study of the UV sensitivity of the biological activity of SV40 DNA (both single- and double-stranded) was started, using normal human cells and Xeroderma cells.

4. SV40-specific RNA

An investigation of the properties of the SV40-specific messenger RNA in productively infected cells has been completed. The messenger RNA species have been characterized with respect of their base sequence and the presence of poly Adenylic acid.

Results of Project No. B 8

Participants:

Dr. A.J. van der Eb

Drs. P.J. Abrahams

Dr. F.L. Graham

Dr. S.O. Warnaar

Title: Cell transformation by oncogenic DNA viruses.

Description of the results:

1. Transforming activity of adenovirus DNA

A new method has been developed to demonstrate infectivity of purified adenovirus 5 DNA. Using this technique, it has also been possible to transform rodent cells with pure viral DNA with a relatively high efficiency. Experiments are now in progress to detect biological activity (e.g. (oncogenic) transformation, induction of tumor antigens) of DNA fragments. In this way it might be possible to isolate those parts of the viral genome which are responsible for transformation. Preliminary experiments suggest that it is indeed possible to transform cells with DNA fragments, obtained by mechanical shear. These transformed cells are being compared with the types of cells obtained upon transformation with UV irradiated adenovirus 5. (Transformation of rat cells with UV irradiated adeno 1 yields at least two types of transformed cells, one of which is transplantable. Adeno 5 and adeno 1 are both non-oncogenic; see also the previous Euratom report.) The possibility that acquisition of oncogenic potential by UV irradiation is due to inactivation of a viral gene will be investigated.

2. Replication of adenovirus DNA

To obtain a better understanding of the interactions of the viral DNA with the host cell, the mechanism of replication of adenovirus 5 DNA in productively infected KB cells was studied. The results have indicated that the replicative intermediates consist of linear, Y-shaped molecules, containing single-stranded DNA at the branching point and often on one of the replicated arms. No evidence has been found for circular intermediates. (The other oncogenic DNA viruses, SV40 and polyoma, contain circular DNA molecules, both within the virion and in the infected cells.)

3. Repair of irradiated viral DNA in mammalian cells

It was suggested from earlier work in this laboratory that the infectivity of single-stranded polyoma virus DNA is much more sensitive towards UV irradiation than that of the double-stranded DNA. This result suggested that a process, similar to excision repair, is operating in mouse cells. To obtain more information about the radiation repair processes in mammalian cells, a similar study has been started, using single-

and double-stranded SV₄₀ DNA and normal human cells as well as radiation sensitive Xeroderma cells as host cells. Since SV₄₀ is able to grow in human cells, but does not produce plaques, it was necessary to develop a method for determining infectivity of SV₄₀ DNA in human cells. Using this technique, the kinetics of UV inactivation of SV₄₀ DNA's are now being determined.

4. Virus-specific RNA's in SV₄₀-infected cells

Two homogeneous virus-specific RNA's are found in SV₄₀-infected BSC-1 cells: (1) a 19S RNA, early in the infection and (2) 19S and 16S RNA, late in the infection. In order to establish the relationship between these species, the various RNA's have been studied with respect of their base composition and base sequence. The results indicated that the early and late 19S RNA's are similar in base composition, and that the 19S and 16S late RNA's have a different base composition and base sequence. This suggests that the 19S and 16S RNA's may contain together the entire genetic information of the viral DNA. In addition, the 19S and 16S RNA's have been shown to contain poly A sequences, like other mRNA's.

Publications:

R.A. Weinberg, S.O. Warnaar and E. Winocour

Isolation and characterization of simian virus 40 ribonucleic acid.
J. Virol. 10, 193 (1972).

A.J. van der Eb

Intermediates in type 5 Adenovirus DNA replication.
Virology, vol. 50, december 1972.

F.L. Graham and A.J. van der Eb

A new technique for the assay of infectivity of human Adenovirus 5 DNA.
Virology 1973.

In preparation:

F.L. Graham and A.J. van der Eb

Transformation of rodent cells with Adenovirus 5 DNA.

S.O. Warnaar and A.W. de Mol

The characterization of two SV₄₀ specific RNA molecules from infected BSC-1 cells.

Contractor: The University College of Swansea

Contract No.: 119-72-1 BIOE

Head of research team(s): Dr. James M. Parry

General subject of Contract: Studies of the genetic,
molecular and adaptive properties of UVS loci in yeast.

The processes which result in cell lethality and the mechanisms of repair of both physical and chemical damage have been studied extensively in bacteria and viruses. This work has provided us with some understanding of the pathways of cellular repair in simple procaryotic organisms.

In order to facilitate the understanding of such processes in eucaryotic organisms investigations are taking place into the action of radiations and chemical mutagens utilizing the yeast Saccharomyces cerevisiae. This yeast provides us with a simple unicellular eucaryotic organism, well characterised genetically, with a chromosomal organisation of its genetic material.

In general terms, the principal projects undertaken during 1972 were:

1. The isolation and characterisation of mutants sensitive to radiations, heat treatment and chemical mutagens.
2. Studies upon the genetic effects of radiation and chemical mutagens with special emphasis upon the induction of mutations and mitotic recombination.
3. Studies upon the behaviour of nucleic acids and proteins after radiation treatment.
4. Analyses of the processes which modify radiation damage and in particular liquid holding recovery after UV irradiation.
5. Studies upon the mechanisms of the regulation of radiation repair, particularly by the use of temperature sensitive mutants.
6. Quantitative and qualitative analysis of the effects of radiation treatment upon nucleic acids and proteins.

Results of Project No. : 1

Head of Project and scientific staff: Dr. James M. Parry

Title of Project: The genetic control of liquid holding recovery and UV light induced "repair resistance" in yeast.

Description of results:

After UV exposure of a yeast culture, liquid holding in non-nutrient solution results in an increase in cell viability compared with a culture plated immediately. After post-UV liquid holding treatment both haploid and diploid yeast cultures show an increase in resistance to a second UV dose range. For example, a control culture and a culture exposed to a UV dose of 4000 ergs/mm² followed by liquid holding treatment required doses of 700 ergs/mm² and 2000 ergs/mm² to produce a 90% reduction in cell viability.

The sensitivity to a second UV dose range has been determined in a range of UV sensitive mutants of yeast which show both positive and negative responses to liquid holding treatment.

Mutants e2 (rad 10) and e32 (rad 21) show increases in cell viabilities after post-UV liquid holding treatment and also show increases resistance to a second UV exposure range. In contrast, the mutants e7 (rad 15), e43 (uvs-5), e5 (rad 3) and e9 (rad 2) all show reductions in cell viabilities after post-UV liquid holding treatment. None of these four mutant cultures showed any increased resistance to a second UV dose range.

Mutants e7, e43, e5 and e9 were crossed to a wild type culture (RAD). The RAD culture was adenine-requiring, showed positive liquid holding and "repair resistance" to a second UV dose range. The resulting diploid cultures were sporulated and tetrad analysis performed. The phenotypes, UV resistance and sensitivity, positive and negative liquid holding, presence and absence of "repair resistance" and adenine independence and requirement show single gene Mendelian segregation in all the spore tetrads analysed. The phenotypes UV resistance, positive liquid holding and "repair resistance" segregated together. The genetical and physiological analysis performed indicates that positive liquid holding and "repair resistance" result from the action of a single gene in each of the mutant cultures.

Mutants e7, e43, e5 and e9 have been shown to be mutant at four separate genes, all of which are involved in the production of the phenotypes' positive liquid holding and "repair resistance".

Results of Project No. : 2

Head of Project and scientific staff: Drs. James M. Parry
Elizabeth M. Parry & R. Waters

Title of Project: Genetic and physiological analysis
of mutants of the genes rad 1 and rad 3 of yeast.

Description of results

Two groups of non-complementing mutants of yeast, sensitive to UV light and comprising 10 and 11 individual isolates respectively have been intensively studied.

Complementation tests and genetic analysis of all pairwise combinations of mutants indicate that the two groups represent alleles of two individual genes, whose products are involved in the repair of UV induced cell damage.

The UV sensitivities were determined for each mutant as haploid, homoallelic (2 copies of the same allele) and heteroallelic (2 different alleles of the same gene) diploid cultures, together with the effects of post-UV modifying treatments such as photoreactivation and liquid holding in non-nutrient solution.

In rad 3 cultures, the order of UV sensitivity of haploid and homoallelic diploid cultures was identical, whereas in rad 1 the order of UV sensitivity shows a number of significant differences. There was no evidence in any of the heteroallelic diploids of complementation producing UV resistance. In each diploid the most resistant allele was dominant to the most sensitive allele.

Considerable variation was found in the post UV liquid holding response of the haploid mutants of both groups. Three characteristic phenotypes were detectable:

- a. liquid holding results in an increase in viability,
- b. liquid holding has no effect on viability,
- c. liquid holding results in a decrease in viability.

The most sensitive mutant cultures show a decrease and the most resistant an increase in viability after liquid holding treatment. In contrast, diploid cultures show only two phenotypes, the most resistant an increase and the most sensitive no change in viability. In heteroallelic diploid cultures the most resistant alleles confer the property of positive liquid holding in the presence of all the sensitive alleles.

Crosses were made between the most sensitive and the most resistant alleles of both rad 1 and rad 3. The resistant rad 1 x resistant rad 3 cultures were more sensitive than the sensitive rad 1 x sensitive rad 3 cultures. The results indicate that the more resistant alleles produce a partially functioning gene product, which results in a reduction in the efficiency of the wild type RAD product. In contrast, the sensitive alleles produced a non-functioning gene product which allowed the maximum expression of the RAD gene product.

The cross-sensitivity of the rad 3 product to gamma , irradiation, heat treatment at 52°, nitrous acid and the alkylating agents ethylmethane sulphonate and methylmethane sulphonate was determined. The mutants were more sensitive to only nitrous acid and in both haploid and diploid cultures they showed the same order of sensitivity to both UV and nitrous acid.

Studies have been initiated into the UV induction of mutation from auxotrophy to prototrophy, fungicide sensitivity to resistance and respiratory sufficiency to dependence in haploid and diploid cultures of rad 1 and rad 3. The initial results indicate a wide variation in the mutation rates of all three markers. The rad 3 cultures all show an increased frequency of prototrophs and resistant cells per unit dose and a lower frequency per viable cell than the RAD culture. The rad 1 cultures show a more variable response, some cultures showing higher and others lower mutation frequency per dose and per survivor. Genetic analysis of the rad 1 mutants indicates that the property of UV sensitivity segregates with the property of increased or decreased mutation frequency.

Results of Project No. : 3

Head of Project and scientific staff: Drs. James M. Parry
and Elizabeth M. Parry

Title of Project: The effects of UV irradiation and
liquid holding post-treatments upon mitotic
recombination in yeast.

Description of results

The exposure of diploid cultures of yeast to both ionising radiations and UV light results in the induction of mitotic recombination both between (intergenic) and within (intragenic) genes. Liquid holding treatment in non-nutrient solution after UV exposure results in increases in cell viability and intragenic recombination together with decreases in intergenic recombination and mutation from auxotrophy to prototrophy.

Post-UV liquid holding treatment also results in an increase in the resistance of the treated culture to a second UV exposure range but not to ionising radiation. This property has been called "repair resistance".

Investigations were made into the effects of a second UV exposure range upon the induction of mitotic recombination. The results shown in Table 1 demonstrate that the frequency of mitotic intragenic recombination is higher per unit of UV exposure during the second UV dose range.

Table 1 The effect of modifying treatment upon the UV-induced frequency of adenine independent recombinants in a heteroallelic culture of yeast. (The results indicate the yields of recombinants produced after UV exposures of 2000 ergs/mm² and at a cell survival of 10%)

Treatment	Increase in adenine recombinants per 10 ⁵ survivors	
	2000 ergs/mm ²	10% survival
Control	105	110
7 days liquid holding	105	110
1320 ergs/mm ² + liquid holding	235	320
2640 ergs/mm ² + liquid holding	300	550

Reconstruction experiments have demonstrated that the increased yield of intragenic recombinants produced during the second UV

exposure range does not result from the differential sensitivity of the recombinant and non-recombinant cells. In contrast, the second UV exposure range produced no increase in the frequency of mitotic intergenic recombinants.

The variation in the induction of the two recombinational events during the second UV exposure range has led us to the postulation that they result from the differential activity of two different and separable repair pathways in yeast.

Results of Project No.: 4

Head of Project and scientific staff: Dr. James M. Parry

Title of Project: Negative liquid holding in yeast.

Description of results:

Negative liquid holding, i.e. the reduction in cell viability produced by liquid holding in non-nutrient solutions after UV exposure is shown by respiratory sufficient cultures of some yeast cultures mutant at the genes rad 1, 2 and 3. These mutants have been shown to be defective in the excision-repair of UV induced pyrimidine dimers. Respiratory dependent cultures show no change in cell viability in non-nutrient solution but do show a reduction when cultures are incubated in glucose solution.

Quantitative studies have been made into the effects upon negative liquid holding of variation in the metabolic condition of the treated cultures and also the frequency of UV induced pyrimidine dimers. Cultures of rad 2 and rad 3 grown for varying periods on solid medium before UV exposure show a wide range of sensitivities. The cultures reach a maximum resistance to UV exposure after five days' growth. In these cultures, irrespective of their UV sensitivity, liquid holding treatment produces the same dose modification factor for the reduction in cell viability. Similar results were obtained when the frequency of UV induced pyrimidine dimers was reduced by exposure to photoreactivating light before liquid holding treatment.

The data suggest that negative liquid holding in excision-deficient mutants of yeast results from a reduction in the activity of an enzyme system involved in the repair of non-photoreactivable UV-induced lesions.

Results of Project No. : 5

Head of Project and scientific staff: Dr. James M. Parry

Title of Project: Liquid holding recovery in UV sensitive diploid cultures of yeast.

Description of results:

Homozygous diploid, UV sensitive cultures were constructed from the haploid mutants e9 (rad 2) and e5 (rad 3). These mutants show a reduction in cell viability when exposed to post-UV liquid holding treatment. In contrast, the diploid cultures show no change in cell viability after liquid holding treatment. The results suggest that in the rad 2 and rad 3 diploid cultures either repair activity is identical before and after liquid holding treatment or the same total repair occurs as a result of the operation of different combinations of enzyme systems.

In order to investigate these two possibilities, diploid cultures of rad 2 and rad 3 were constructed in which the frequency of mitotic intergenic recombination and mutation was scorable before and after post-UV liquid holding treatment. It was shown that the frequencies of both intergenic recombinants and mutations to prototrophy were reduced by a period of post-UV liquid holding even though viability remains constant. Reconstruction experiments were performed with non-recombinant and recombinant cells which demonstrate that the observed changes in recombination frequency do not result from the differential sensitivity of the two cell types.

The results indicate that changes occur in the relationship between the repair enzymes of rad 2 and rad 3 diploids of yeast during liquid holding treatment and that these changes are expressed as changes in the yields of recombinant and mutant cells but not in the total yield of viable cells.

Results of Project No. : 6

Head of Project and Scientific staff: Drs. Elizabeth M. Parry and James M. Parry

Title of Project: Temperature sensitive repair of UV light and ionising radiation damage in yeast.

Description of results:

A major problem in the furthering of our understanding of cellular repair in yeast relates to our lack of control over the cellular processes which occur after the induction of nucleic acid damage.

We have undertaken the isolation of mutant cultures which have the secondary restriction of temperature sensitivity upon cellular repair, i.e. the induction by mutagen treatment of a lesion in a gene coding for a repair enzyme which results in a functional enzyme under permissive conditions (28°), but leads to a defective enzyme under restrictive conditions (37°).

Approximately 200 mutants showing the required phenotype were isolated and have been assigned the prefix ts-rad. The mutants were isolated on the basis of their sensitivity to UV light but a number were cross-sensitive to gamma irradiation and alkylating agents.

Each mutant was mated to a wild type culture of opposite mating type and tetrads analysed from the resulting diploid. This genetic analysis indicated that each ts-rad phenotype behaved as a single Mendelian gene. Complementation tests were performed between the individual ts-rad mutants and the previously characterised rad loci. The results have demonstrated that a number of the ts-rad mutants have been isolated at the three rad loci 1, 3 and 16.

The effects of the ts-rad phenotype upon macromolecular synthesis has been determined after UV and gamma irradiation. These experiments have enabled us to elucidate the effects of a number of the ts-rad mutations upon nucleic acid and protein synthesis under permissive and restrictive conditions.

The time of action after radiation treatment of a number of the genes defective at 37° has been determined by the use of temperature switch experiments.

One of the mutants isolated, ts-rad 503 also shows a temperature sensitive phenotype with regard to spontaneous mutation. When grown at 28°, cultures of this mutant produce prototrophic mutations at six auxotrophic loci. In contrast, when grown at 37° no back mutation is detectable. At least 90% of the prototrophic mutants produced at 28° result from mutation at suppressor loci.

Results of Project No. : 7

Head of Project and scientific staff: Drs. Elizabeth M. Parry and James M. Parry

Title of Project: The regulation of the repair of UV light and gamma ray damage in yeast.

Description of results:

A diploid culture of yeast has been isolated which lacks the characteristic resistant shoulder of the survival curve shown by yeast cultures after low doses of UV light and gamma irradiation. After sporulation of this culture the UV sensitivity of the resulting haploid cultures was determined in detail at a wide range of UV exposures. The haploid cultures show the segregation of a new phenotype, i.e. a U-shaped survival curve with a period of extreme sensitivity at low doses followed by a period where cell viability increases with increased UV exposure. An example of this type of survival curve compared to that of a wild type culture is shown in Figure 1.

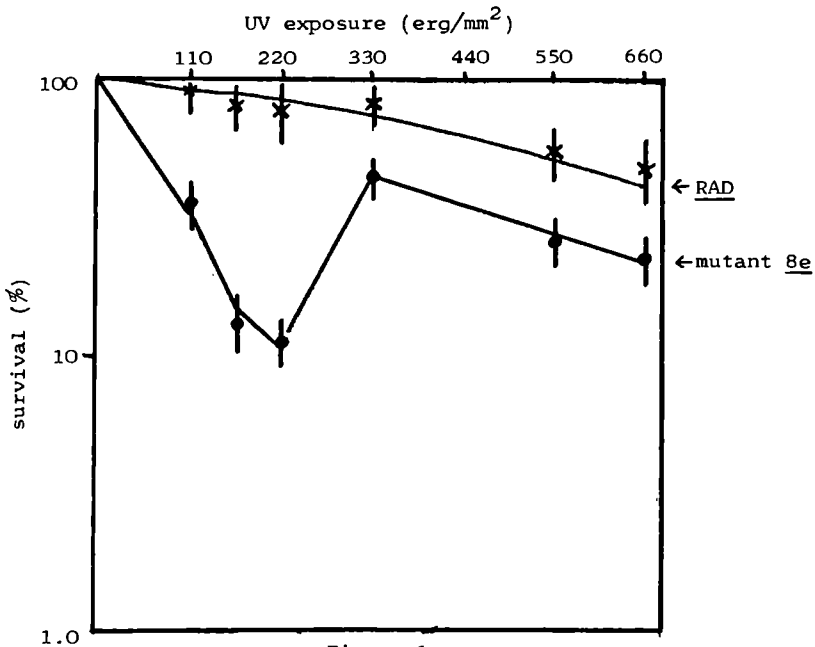


Figure 1

The U-shaped phenotype is recessive and segregates as a single Mendelian gene. The position of the U-shaped portion of the survival curve is variable in different isolates and appears to depend upon the genetic background of the treated culture. In homozygous diploid cultures the U-shaped portion of the curve is detectable at higher UV exposures.

The U-shaped phenotype has been studied by the use of a number of treatments:

- a. variation in UV dose rate,
- b. treatment with photoreactivating light,
- c. plating on medium containing caffeine.

The results demonstrate that the position of the U-shaped portion of the survival curve is independent of the dose rate and depends upon the total UV exposure. After photoreactivating light treatment, viability is increased at high UV exposures but the U-shaped phenotype is still observed at low doses. Treatment with caffeine results in the elimination of the rise in viability with dose produced during the U-shaped portion of the survival curve.

The results suggest that the U-shaped phenotype represents a defective regulator gene which is dose-dependent and may be involved in the operation of the excision-repair enzyme system which has been shown to operate after UV exposure in yeast.

Results of Project No. : 8

Head of Project and scientific staff: Dr. James M. Parry,
Mr. W. E. Evans

Title of Project: The cross-sensitivity to radiations,
chemical mutagens and heat treatment of a number of
mutant cultures of yeast.

Description of results:

Mutants of yeast, isolated on the basis of their sensitivity to ionising radiations, prefixed rad 50, 51, 52 and 53 were tested for their sensitivity to heat treatment (at 52°), chemical mutagens and UV light. All the primarily X-ray sensitive mutants tested were also cross-sensitive to heat treatment and alkylating agents whereas UV sensitive mutants of rad 1, rad 2 and rad 3 show wild type (RAD) sensitivity to heat treatment.

A number of mutants were isolated on the basis of their sensitivity to heat treatment and were shown to be cross-sensitive to ionising radiations and alkylating agents. Genetic analysis indicates that in a number of the heat sensitive mutants, the heat sensitive phenotype is dominant in heterozygous diploid cultures and interferes with the process of sporulation in such cultures.

A third group of mutants were isolated, which show UV sensitivity at high UV exposures, even after photoreactivating light treatment. These mutants are presumed to represent cultures defective in the repair of UV light damage other than pyrimidine dimers. Mutants of this phenotype also show cross-sensitivity to ionising radiations, heat treatment at 52° and alkylating agents.

All the various types of mutants have been tested for liquid holding recovery after the different inactivating agents. The results indicate a considerable variation in the effects of liquid holding, both increases and decreases in viability were detectable.

The results indicate that the damage produced by ionising radiation, heat treatment, alkylating agents and a fraction of the UV light damage are repairable by a single repair pathway in yeast.

Results of Project No. : 9

Head of Project and scientific staff: Dr. C. E. Deutch

Title of Project: The effects of radiation exposure
upon the synthesis of macromolecules in Saccharomyces
cerevisiae.

Description of results:

The amounts of DNA, RNA, and protein per cell are being measured during balanced, exponential growth of yeast. Experimental procedures have been established for the accurate extraction and assay of these macromolecules. The rate of division is being varied by the use of media of varying richness. Preliminary results indicate that, in a rich medium permitting a relatively high growth rate, the amounts of RNA and protein are increased two to three times above the levels observed in stationary-phase cells. However, the DNA content is only slightly greater than the stationary-phase level. This type of data will be used to develop models for the regulation of macromolecular synthesis in this simple eucaryote. Since the enzymes for DNA synthesis appear to be synthesized cyclically before each \underline{s} period, a comparison of the radiation-sensitivity of the cells grown in the various media and an analysis of the effect of radiation treatment on subsequent growth should then provide a basis for assessing the role of these enzymes in repair processes.

Results of Project No. : 10

Head of Project and scientific staff: Dr. James M. Parry,
Roger S. Briggs, Peter J. Davies.

Title of Project: The screening of environmental
chemicals for possible mutagenic activity.

Description of results:

Unlike radiations, whose mutagenic activity has been studied for over forty years, only a relatively small fraction of the chemicals present in our environment have received similar study. In order to evaluate the hazards involved in the use of possibly mutagenic chemicals a number of different test systems must be utilized to provide the necessary information.

The yeast Saccharomyces cerevisiae, provides us with a convenient eucaryotic organism for the detection of genetic activity. The organism is easily and quickly cultured and a range of gene effects may be detected. These effects include, mutation at both chromosomal and cytoplasmic loci, meiotic and mitotic recombination both between (intergenic) and within (intragenic) genes and changes in chromosome number. Work is in progress into the development of a range of yeast cultures capable of detecting such effects after mutagen exposure.

Mitotic intragenic recombination (gene conversion) has been used as a non-specific genetic event in the screening of a number of environmental chemicals such as herbicides, insecticides and some pharmaceutical products. This work has indicated that the herbicides mecoprop, dinoseb and paraquat, and the insecticides lindane and formathion show genetic activity in the yeast system.

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Contract N. 111-72-1 B10I

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 experimental data on a large scale in eucaryotic organisms on the distribution of the molecular types of mutations induced by x-radiations, to investigate the variations of mutagenesis spectrum in relation to the dose and to the nuclear stage. The research was carried out on two different organisms (Sacch.cerevisiae and Schiz. pombe) showing each one peculiar and useful properties in their life cycle.

In Sacch.cerevisiae it was previously shown that X-radiation can induce both base substitutions and insertion/deletion mutations. The main activity during the last year was concerned with a possible change of x-radiations molecular specificity with the dose. Evidence was obtained that the frequency of temperature sensitive lethals (theoretically supposed to be due to base substitution) is increasing with the dose from 20 to 120 Krad. On the contrary the frequency of nonsense mutations (certainly due to base substitution remain constant within the same range of doses. Further investigation are required to interpret such discrepancy. With the Schiz.pombe during the first period of researches inactivation curves for wild type (G_1 and G_2 cells) and for three different repair-deficient strains have been obtained. A rather large number of forward mutations in two adenine loci has been obtained at different X-ray doses in the wild type (G_1 cells) and in a repair deficient strain (G_2 cells). A preliminary investigation of the molecular nature of about 200 forward mutants at the above loci was carried out.

Project N. 1

Prof.G.E.Magni,Prof.G.P.Sironi,Dr.S.Sora

Molecular nature of point mutations induced by X-radiations in
Sacch. cerevisiae

Progress report

In the previous annual report it was mentioned that some preliminary data showed a possible change of specificity of x-radiations with the dose. During the year dealing with this report our major aim was to explore deeply this presumed change of specificity,

a) Conditioned lethals

We first tried to expand the preliminary evidence on the frequency of conditioned lethals(c.l.). Conditioned lethals are mutants able to grow at permissive temperature(in our experiments 24°C)and unable to grow at non-permissive temperature(34°C). Whatever is the mutated gene it is generally believed that its product is normally synthesized but its structure is changes. It is therefore generally accepted that this kind of mutants are due to base substitutions leading to a change of one aminoacid in the polypeptide chain. On the other hand conventional auxotrophs can be due to either to base substitutions, leading to missense or nonsense mutants, or to frameshifts. Considering therefore that :

Conditioned lethals = missense

Auxotrophs = missense + nonsense + frameshift

The ration c.l./auxotrophs must indicate the relative efficiency of a given treatment in inducing missenses.

During the last year two independent complete dose response curves were obtained for induced conditioned lethals an auxotrophs. Our preliminary observation seems to be fully confirmed. The relative frequency of conditioned lethals increases with the dose from about 6% at 20 Krad to 25% at 160 Krad. Accepting the above theoretical consideration one should state that mutagenic

specificity of X-radiations changes with the dose.

b) Nonsense mutants

Before indulging in speculations on the possible causes of the observed "change of specificity" it was considered mandatory to perform a more severe test. No doubt is today possible on the fact that nonsense mutants can arise only from a base substitution. It was therefore thought that the estimate of the relative frequencies of nonsense mutants and auxotrophs should give more reliable informations on the possible change of specificity. All the auxotrophs of the previous experiments were tested for the presence of nonsenses. No systematic variation of NS frequency is observed over a wide range of doses from 20 Krad to 160 Krad. The interpretation of the discrepancy between the two experiments is not easy. In molecular terms the second experiment (induction of NS) is obviously more reliable and therefore we are inclined to accept the idea that X-radiations do not show any significant change of molecular specificity with the dose.

The effect observed in the first experiment can be accordingly interpreted as follows. Deletions of entire triplets can operationally behave as base substitutions and consequently as conditioned lethals.

Project N. 2

Prof. N.Loprieno and Dr. A.Abbondandolo, Dr. S.Baroncelli, Dr. M.Simili, Dr. R.Barale

Molecular nature of point mutations induced by X-radiations in Schiz.pombe.

Progress report

The survival curves of G_1 and G_2 cells of the wild type strain of S.pombe after X-ray treatments present a different kinetic, namely G_2 cells are more sensitive to the inactivation, a result different from that produced by treatment with UV light; the preliminary experiments on the induction of forward-mutations at two adenine dependent loci have shown a higher sensitivity from the G_1 cells at least at the doses already analyzed which correspond at a survival level of 20-30%. 187 purple mutants ($ade6^-$ and $ade7^-$) have been isolated from G_1 X-ray treated cells: for two X-ray doses there is an increase with the dose in the percentage of the mosaic mutant colonies. This finding does indicate the production of alterations in one single strand of DNA, according to the data obtained with other mutagens (Abbondandolo & Bonatti, 1970; Abbondandolo & Simi, 1971). No substantial differences between two different treatments by X-ray have been noticed about the molecular nature of mutant isolated: in both the treatment an average of about 45% of mutants have resulted from base-pair substitutions. Experiments done with repair-deficient strains specific for UV inactivation or for both UV and X-rays have shown that a production of reverse-mutation in the $ade7-152$ strain of S.pombe is relatively increased in these particular strains, although the curve of the ratio K/Ko is lower in one of these strains (uvs10-198) compared with the wild type (uvs⁺), due to the large fraction of spontaneous reversion occurring

in this particular strain (Loprieno, 1973); some 300 purple mutants (mutation at the ade6 or ade7 loci) have been already isolated from three different treatment of X-rays and genetic and physiological analyses are in progress at the present.

N. Loprieno has presented a communication with the title "On the possible involvement of repair on the process of mutations in S.pombe" (Radiation Research, 51, 497-98, 1972) at the Symposium "Fundamental Aspects of Mutagenesis and Repair in Eucaryotes" held during the 20th Annual Meeting of the U.S. Radiation Research Society (Portland, Oregon, U.S.A., May 14-18, 1972).

Contractant de la Commission : Université Libre de Bruxelles
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.

The present report summarizes the work done in 5 different (but closely linked) laboratories which are studying the effects of radiations on biological systems which vary in complexity from nucleic acids and bacteriophages to rabbits.
The main results are the following :

I. Nucleic acids.

ESR observations have been performed on mixed aggregates of tryptamine or histamine and derivatives of DNA or proflavine after γ irradiation. These results indicate the occurrence between these components of spin transfers depending on their stacking interactions and their electron affinities.

II + III. Micro-organisms and bacteriophages.

Work on λ phage and its DNA has allowed an analysis of the protection by a flavin against γ irradiation and has shown that there must be common pathways between UV reactivation and UV mutagenesis. A dimer of bacteriophage Mu can insert, at any point between the bacterial chromosome and an infecting λ phage : it can thus link together two completely unrelated DNA sequences.

In order to induce the development of a λ prophage with or without immunity, a positive regulatory produced by the N gene is needed.

The mutagenic effects of tritiated water on bacteria have been studied, as well as the effects of gal-transducing viral particles on human galactosemic cells.

IV. Cells and embryos

It has been shown that single strand breaks in DNA after UV irradiation are repaired by the insertion of 40 nucleotides; this kind of irradiation increases the number of DNA exchanges between sister chromatids, a phenomenon which seems closely related to UV radiosensitivity and probably repair synthesis of DNA.

Synkaryons between 2 strains of chinese hamster fibroblasts have been studied : no marked difference in X-rays and UV radiosensitivity between the somatic hybrids and the parental cells has been observed so far. After UV, repair is more inhibited by cafein in the hybrids than in the parental cells.

In amphibian eggs, the most sensitive steps to X-irradiation is probably the activity or the synthesis of thymidinekinase.

X-rays inhibit DNA and RNA synthesis, but increase protein synthesis in glioma cells. They produce a strong increase in length of the cytoplasmic processes; this increase is inhibited by colchicin, but not by cycloheximide.

V. Immunological studies with irradiated rabbits.

A large number of experiments have led to the following tentative conclusions : antibodies are synthesized by host radioresistant cells. There is probably an information transfer between memory cells and antibody producing cells.

Toluant cells seem to be able to synthesize inhibitors for the expression of new clones of the same immunological specificity.

Progress has been made in attempts to isolate the mRNA for anti-TMV antibodies.

Project Nr I.

PRIMARY EFFECTS OF RADIATION ON NUCLEIC ACIDS

A. Bertinchamps, S. Gregoli, M. Olast and M. De Vré.

We have shown in previous work on the γ radiolysis of DNA and DNA nucleotides complexes that the radiation damage, as observed by ESR, is redistributed in such a way that the final radical population is very different from the initial one. The redistribution proceeds via the stacking of the basis towards the molecules of highest electron affinity. This stacking interaction, far from belonging specifically to the nucleotides is very frequent between numerous molecules in the living state and in the course of certain pharmacological interactions. We have therefore studied if the phenomenon of spin migration which we observed previously in the complexes between nucleotides would also occur in other stacking complexes with alternance between molecules of different electron affinity. We observed by ESR the behavior of mixed aggregates of tryptamine or histamine and derivatives of DNA or proflavine after γ irradiation. Tryptamine and histamine are better donors and proflavine is a better acceptor than the various DNA derivatives.

The results indicate the occurrence after γ irradiation of a spin transfer from histamine towards the four nucleotides of DNA as well as from tryptamine towards the four nucleosides and proflavine. They complete and confirm the mechanism proposed previously. They indicate that both the stacking and the difference in electron affinity are instrumental for the transfer. Furthermore, there is evidence that the degree of stacking between amino acids and pyrimidine bases as well as the relative difference in electron affinity play a crucial role in determining the intensity of the transfer in their stacked molecular aggregates. The estimation of the contribution of each of these two factors is not feasible because of their mutually coordinated effect on the transfer process; with a view to achieving a more profound insight into the interplay of stacking and electron affinity we have studied their effect on the extend of molecular interactions in aqueous solutions of pyrimidine nucleosides and 5-halo-nucleosides with a good electron donor molecule : serotonin (5-hydroxytryptamine) by NMR spectroscopy.

The results show that the interaction ability of nucleosides is enhanced when the electron deficient character of the base rings is increased by introducing a 5-halogen atom or by ring protonation in the case of cytidine, i.e., the electron affinity differences of base rings in a series of pyrimidine nucleosides play an important role in determining their relative interaction abilities with a given donor.

Finally, we have initiated the study by ESR of the γ radiolysis of the constituents of nucleic acids in frozen aqueous solution.

Compared to the material which we used before (polycrystalline powders) the frozen solutions must allow us to tackle the problem of the relative amount of direct and indirect effects. Moreover they permit, by a progressive warming of the sample, to follow the kinetics of a large number of radicals reactions. Preliminary results indicate that the products of the radiolysis of water do not, in these experimental conditions, play any role in the sequence of radicals reactions occurring at the level of the solute molecules.

Publications

Free radical distribution in gamma-irradiated DNA

S. GREGOLI and A. BERTINCHAMPS

Int. J. Radiation Biol., 1972, Vol. 21 n° 1, 65-73

Computer analysis and reconstruction of E.S.R. spectra of gamma-irradiated DNA

S. GREGOLI and A. BERTINCHAMPS

Int. J. Radiation Biol., 1972, Vol. 21, n° 1, 75-85

Energy transfer in gamma-irradiated DNA as studied by Electron Spin Resonance

Thesis by S. GREGOLI

June 1972.

An NMR study of the relative interaction abilities of different Pyrimidine Nucleosides with Serotonin

R. Mathur-DE VRE and A. BERTINCHAMPS.

Submitted for publication to Biochemica Biophysica Acta.

Electron transfer in γ irradiated complexes between aromatic amino acids and DNA derivatives

M. OLAST and A. BERTINCHAMPS.

Submitted for publication to Biochemica Biophysica Acta.

Communication

Gamma irradiation of complexes between amino acids and DNA derivatives

M. OLAST and A. BERTINCHAMPS.

Proceedings of the 9th Annual meeting of the European Society for Radiation Biology. p. 123, 26-28.9.1972.

Résultats du projet n° II

Chef du projet et collaborateurs scientifiques :
M. Errera, M. Brunfaut, P. Caillet-Fauquet, M. Defais,
J. Leys-Mercenier, G. Michel-Maenhaut, A. Miller-
Faurès, J. Rommelaere, B. Srivastava, M. Susskind,
N. Vanderloo-Henry, M.F. Van Horen-Bourguignon

Titre du projet : Mécanismes de la réparation du
DNA chez les microorganismes (E.coli - λ) et
les cellules de mammifères

I. Microorganisms

a) - The *uvrA* and *polA* genes of *E.coli* as well as the red function of phage λ play a significant role on the survival of γ irradiated phage. Alcal. sucrose gradient analysis suggests that mutations in *polA* and red delay the repair of single strand breaks and gaps (B. Srivastava).

- Proflavine protects phage λ or its DNA against direct as well as indirect effects of γ rays (irradiation at -190°C and 0°C) probably by decreasing the formation of anionic radicals in DNA bases and by scavenging hydrated electrons (appearing in the solvent). This could be achieved either by dye intercalated between DNA bases or bound ionically to DNA and by dye in solution. Identification by electron microscopy of possible breakage of the DNA at specific sites is in progress, as well as the possible repair pathways of the remaining damage (G. Michel-Maenhaut).

b) UV reactivation of phage λ in a *polA* mutant of *E.coli* remains effective if assayed at UV doses to *E.coli* sufficiently low (P. Caillet-Fauquet and M. Defais).

c) Induced reactivation of phage λ irradiated by UV can be obtained by irradiating *E.coli* by γ rays instead of UV ; induction is dependent on the *recA* function. Attempts to inhibit induced reactivation (by γ rays and by UV) by chloramphenicol are in progress. The study of individual infective centers indicates that UV reactivation leads to an increase in the number of bacteria susceptible to support multiplication of UV irradiated phage (M. Defais and P. Caillet-Fauquet).

d) Low temperature (25°C) kinetics of λ DNA attachment to the bacterial membrane and its dependency on gene N suggest that the N product probably stabilizes the DNA-membrane complex after its formation. This study may be valuable in approaching the mechanism of coordination of various repair systems (M.F. Van Horen-Bourguignon, N. Vanderloo-Henry, J. Leys-Mercenier).

e) The *polA* mutation in *E.coli* does not prevent the appearance of UV induced clear plaque mutants in phage λ ; these experiments and those mentioned in b) support the hypothesis that UV reactivation and UV mutagenesis have some common pathways (P. Caillet-Fauquet and M. Defais).

II. Mammalian cells

a) A chinese hamster radiosensitive mutant (V79/79) and several *Xeroderma pigmentosum* lines are under study with the aim of finding out if there is a correlation between radiosensitivity and the frequency of sister chromatid exchanges - such exchanges could indicate the existence of a recombination type of repair (see d) (M. Brunfaut, M. Susskind).

b) Unscheduled DNA synthesis and repair replication after UV irradiation have been studied in normal chinese hamster cells and in cells in which thymine has been substituted with BUDR. There is a good quantitative correlation between both repair mechanisms and the cytochemical and biochemical results show that each single strand break is repaired by the insertion of 30-40 bases in the damaged strand (J. Rommelaere, A. Miller-Faurès)

c) Search for a radioinduced repair mechanism : does the survival of an irradiated virus increase if plated on irradiated cells ? Technical problems and shortage of time have so far hindered the progress of this line of research

d) - Synkarions between 2 deficient mutants of chinese hamster cells have been cloned and their chromosomes studied : a few chromosomes are lost in the first few months and the chromosome number has remained \pm 38 (instead of 46) for almost two years (M. Brunfaut, M. Susskind, J. Rommelaere)

- Such synkarions have a UV radiosensitivity similar to either of the parent strains, but repair after small doses is inhibited by caffeine to a greater extent in synkarions suggesting that tetraploid cells are more sensitive to some kind of damage (dominant lethals ?) than diploid cells but which is more efficiently repaired in the tetraploid cells in absence of caffeine (J. Rommelaere)

- DNA exchanges between sister chromatids of diploid cells and between chromosomes of tetraploid cells have been detected by radioautographic and biochemical methods. These exchanges increase after low doses of UV irradiation. Their possible relationship to DNA repair is under study (J. Rommelaere, M. Susskind, M. Brunfaut).

A general review on "the stability and evolution of DNA in the light of molecular radiobiology" has just been written (J. Rommelaere, M. Radman, M. Errera)

Publications

P. CAILLET-FAUQUET and M. DEFAIS, UV reactivation of phage λ in a polA mutant of E. coli, *Mutation Res.*, 15, 353-355 (1972)
Comm. Luntenen Lectures Mol. Genetics, sept. 1972

G. MAENHAUT-MICHEL, Radioprotection of proflavine against direct and indirect effects on DNA, *Comm. 9th Ann. Meeting Eur. Soc. Rad. Biol.*, Rome, sept. 1972

B. SRIVASTAVA, Repair in phage λ irradiated to γ -rays, *Comm. Luntenen Lectures Mol. Genetics*, sept. 1972

J. ROMMELAERE and M. ERRERA, The effect of caffeine on the survival of UV-irradiated diploid and tetraploid chinese-hamster cells, *Int. J. Rad. Biol.*, 22, 285-291 (1972)

N. HENRY-VAN DER LOO, J. MERCENIER-LEYS, M.F. BOURGUIGNON-VAN HOREN and M. ERRERA, Association du CNA phagique avec la membrane bactérienne lors de l'infection d'une bactérie sensible par λ , *Arch. Int. Physiol. Biochem.*, 80, 603 (1972)

J. ROMMELAERE, M. SUSSKIND and M. ERRERA, Chromosome and chromatid exchanges in chinese hamster cells, *Chromosoma* (in press)

J. ROMMELAERE and M. ERRERA, Synthesis and repair replication in chinese hamster cells, *Arch. Intern. Physiol. Bioch.* (in press)

M. RADMAN, J. ROMMELAERE and M. ERRERA, Stability and evolution of DNA from the point of view of molecular radiobiology, in "Physicochemical properties of DNA", ed. by J. Duchesne, Acad. Press (in preparation)

M. ERRERA, *Biologie moléculaire et médecine*, Bruxelles-Médical, 52^e année, n°2, 81-87 (1972)

Résultats du projet n°III

Chef du projet et collaborateurs scientifiques : R. THOMAS
and collaborators (see report)

Titre du projet : The establishment and stability of the state
of provirus : genetic factors and effects of physical agents

1. Mechanism of provirus integration

(mammalian cells : S. Mousset, in collaboration with F. Couzin, Institut Pasteur de Paris ; bacterial cells : A. Toussaint, M. Faelen, M. Couturier and F. Van Vliet)

- a) (in collaboration with several other laboratories : Rijswijk, MIT, Cold Spring Harbor, San Diego)

Bacteriophage Mu inserts in any DNA sequence, but through a defined site (attachment site) of its own genome - apparently, the ends of the vegetative chromosome (papers from the different laboratories sent jointly to Virology).

b) A dimer of bacteriophage Mu can insert, through one of its attachment sites anywhere in the bacterial chromosome, through its other attachment site, anywhere in a coinfecting bacteriophage λ ; this results in insertion of the latter in any permutation, sandwiched between two Mu (Toussaint and Faelen, Nature, in press). This provides in principle a way to connect any two DNA sequences, even if they are completely unrelated.

c) Attempts to demonstrate a mutagenic or transforming effect of phage Mu in mammalian cells have failed so far, but will be repeated with more efficient techniques. Human galactosemic fibroblasts have been transformed with SV40, in connection with the research described in § 4.

2. Induction of development of prophage with or without immunity

(R. Thomas, C. Dambly, J. De Lafonteyne)

a) Even in the absence of immunity, most of the prophage genes remain silent unless one provides a positive regulator protein - the product of gene N. Even in the presence of immunity, the late genes can be switched by another positive regulator - the Q product (Couturier, Dambly and Thomas, Molec. Gen. Genetics, 1972, in press).

b) C. Dambly has shown in collaboration with Dr. Stevens (Rijswijk) that "paradoxical" differences in the behaviours of the related phages λ and 21, are due to the presence of a strong rightwards promoter in the latter.

c) A method for describing, and treating complex regulatory circuits in boolean terms has been developed by R. Thomas (manuscript sent to J. Theor. Biol.)

3. Mutagenic effect of $^3\text{H-H}_2\text{O}$ in bacteria (N. Wantens)

N. Wantens observes a very significant increase of the frequency of Streptomycin-resistant mutations in E.coli K12 after incubation in a medium containing $^3\text{H}_2\text{O}$ (300 $\mu\text{C/ml}$).

4. Effect of gal-transducing viral particles on human galactosemic cells
(A. Résibois and J. De Lafonteyne)

It is well known that at least one other laboratory has not been able to repeat Merrill's experiments and that himself has some difficulties in repeating them. However, the results of Résibois and De Lafonteyne are rather encouraging in the sense that, in precisely defined conditions, they have found repeatedly a significant improvement of the survival of the galactosemic cells in the presence of galactose, after treatment with λgal . Now that the proper conditions seem to have been found it should be possible to analyze the phenomenon at the enzymatic level and check whether, in some cells at least, the provirus has been integrated.

Publications

- SZPIRER, J. Le contrôle du développement des bactériophages tempérés IV. Action spécifique du produit N au niveau d'une barrière de transcription. *Molec. Gen. Genetics*, 113, 297-304 (1972)
- GHYSEN, A. and PIRONIO, M. Relationship between the N function of bacteriophage λ and host RNA polymerase. *J. Mol. Biol.*, 65, 259-272 (1972)
- BOLLEN, A., PETRE, J. and GROSJEAN, H. Direct biochemical approach to the structural heterogeneity of 30S ribosomes from *E.coli*. *FEBS Letters*, 24 327-330 (1972)
- CASTELLAZZI, M., BRACHET, P. and EISEN, H. Isolation and characterization of deletions in bacteriophage λ residing as prophage in *E.coli* K12. *Molec. Gen. Genetics*, 117, 211-218 (1972)
- PETRE, J., BOLLEN, A., NOKIN, P. and GROSJEAN, H. The binding of *E.coli* ribosomes to matrix bound messenger RNA. *Biochimie*, 54, 823-827 (1972)

In press

- COUTURIER, M., DAMBLY, C. and THOMAS, R. Control of development in temperate bacteriophages V. Sequential activation of viral functions *Molec. Gen. Genetics*
- TOUSSAINT, A. and FAELEN, M. Mu dimers promote random integration of λ -gal phages in the chromosome of *E.coli*. *Nature*
- LECOCCQ, J.P. Aspects génétiques et biochimiques de la régulation de la transcription chez la bactérie *E.coli* et ses phages. *Biochimie*
- DE WILDE, M. Caractérisation d'une mutation de résistance à la Spectinomycine. *Arch. Intern. Physiol. Bioch.*
- LECOCCQ, J.P. and DAMBLY, C. Effets d'une mutation affectant la sous-unité β de la RNA polymérase de la bactérie *E.coli* sur le comportement du phage λ . *Arch. Intern. Physiol. Bioch.*

Submitted

- THOMAS, R.
Boolean formalization of genetic control circuits (sent to *J.Theor.Biol.*)
- FAELEN, M. and TOUSSAINT, A.
Isolation of conditional defective mutants and characterization of the prophage map of phage Mu-1 (sent to *Virology*)
- BOLLEN, A., FAELEN, M., LECOCCQ, J.P., HERZOG, A., ZENGEL, J., KAHAN, L. and NOMURA, M.
The structural gene for the ribosomal protein S18 in *E.coli* I. Genetic studies on a mutant having an alteration in the protein S18 (sent to *J. Mol. Biol.*)
- KAHAN, L., ZENGEL, J., NOMURA, M., BOLLEN, A. and HERZOG, A.
The structural gene for the ribosomal protein S18 in *E.coli* II. Chemical studies on the protein S18 having an altered electrophoretic mobility (sent to *J. Mol. Biol.*)

Résultats du projet n° IV

Chef du projet et collaborateurs scientifiques:
J. Brachet. Collaborators : H. Alexandre,
V. Heilporn, A. Lievens, S. Limbosch,
F. Zampetti.

Titre du projet :

1. - Radiosensitivity of the early stages of embryogenesis.
 2. - Morphological modifications of rat glial tumor cells after X-irradiation.
- Radiosensitivity of a synkaryon obtained by fusion.
-

1. a. Study of the highly radiosensitive metabolic pathway leading to the incorporation of ^3H uridine into DNA in amphibian eggs (H. Alexandre).

We have previously demonstrated, by both autoradiography and biochemical methods, the high radiosensitivity of ^3H uridine incorporation into DNA during cleavage of amphibian eggs. Since this process involves the reduction of ribose to d-ribose, we have first tried to isolate ribonucleotide reductase from the eggs. Unfortunately, the reproducibility was not satisfactory and we had to use a more indirect approach.

In order to establish the exact site of action of the ionizing radiations, we have used deoxyuridine (dU) as the precursor for DNA synthesis. This allows a study of the radiosensitivity of the dUMP into dTMP methylation a step which is catalyzed by thymidylate synthetase.

Autoradiographs have clearly shown that the incorporation of dU is small as compared to that of thymidine into DNA at the morula-young blastula stages of Pleurodeles. It is as radiosensitive as ^3H -uridine utilization for DNA synthesis, being already inhibited after 500R. We may therefore conclude that irradiation acts at these stages mainly, if not exclusively, on the activity or synthesis of thymidylate synthetase.

b. In vitro culture of preimplantation mouse embryos (H. Alexandre).

This is a pre-requisite for the analysis of radiosensitivity at early stages of mammalian development.

We are trying to obtain optimal conditions for the culture of mouse ova according to the method of Whittingham (Physiological Laboratory, Cambridge, U.K.), where we stayed during one week.

Random-bred albino, Balb/c⁺ and AKR mice have been used. Virgin females of 8-10 weeks old were mated and ova or embryos at different developmental stages could be obtained, at various times after mating, by perfusion of the oviducts with a small volume of medium. We are mainly interested in obtaining 2 cell stages in order to study the radiosensitivity of this early stage of development.

The yield of embryos was increased considerably (up to 40 ova mouse) by prior treatment with gonadotrophins (intraperitoneal injection of 10 i.u. of pregnant mare's serum (PMS) gonadotrophin, and human chorionic gonadotrophin (HCG) 2 days later).

Cytochemical examinations have been performed at regular intervals during the period of time between the first injection and 15 hrs after the second. Injection of PMS induced maturation and ovulation of

several oocytes. Injection of HCG induced a greater number of oocytes to continue meiosis (from the dictyate stage to metaphase II) during the first 6 hrs. Ovulation usually followed.

We are now able to obtain a successful in vitro development from the 2 cell stage to blastocyst in a chemically defined medium based (modified Krebs-Ringer, supplemented with lactate, pyruvate, glucose, crystalline bovine serum albumin and antibiotics).

The importance of using distilled water of the highest purity should be emphasized : with bidistilled water, we obtain 35% of development only, whereas with tridistilled water (Difco), we have obtained 100% blastocysts, starting from 4 cell stages. Mistakes in the interpretation of the results, in irradiation experiments, could easily be made if the culture conditions are not very precisely standardized.

2. a. Morphological modifications of rat glial tumor cells after X-irradiation. (V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti).

It has been recently observed that X-irradiation of in vitro cultivated mouse neuroblastoma cells induces morphological differentiation, as evidenced by the formation of axons (Prasad, 1971). In order to find out whether similar phenomena can be obtained when other nervous tumor cells are treated in the same way, the effects of X-irradiation on rat glial cells have been observed by optical and electron microscopy. Nucleic acid and protein synthesis has also been studied in control and irradiated cells by radioautography. Glial tumor cells were derived from chemically induced rat brain tumor (Benda, 1971). Since plating efficiency of glial cells is very low, a different method was used for the establishment of survival curves : cell viability, after X-irradiation was measured by counting the cells in definite areas of a small culture cell chamber, every 24 hours, for several days. Irradiation conditions were : 50kV, 25mA, filtration with 1 mm aluminium : the dose rate was 300 R/min. The cells were irradiated without cover protection, at room t° after removing the culture medium. High doses of X-rays (from 900R to 2000R) provoke a general cell death; the most interesting observations have been obtained after a 300R and 600R irradiation. During the three days following 300R irradiation, cell division is delayed as shown by both the presence of a lag phase in the growth curve and a diminution in the % of cells incorporating ³H thymidine. While DNA and RNA syntheses decrease after irradiation, protein synthesis is stimulated. The striking cytological effects of X-rays are an enlargement of the cells, an increase in the number of giant multinucleated cells and especially a morphological modification shown by the elongation of cytoplasmic processes. The % of cells with extrusions longer than 40 um changes from 5% in the control population to more than 50% in the irradiated one. Cycloheximide, at a dose (1 ug/ml) which strongly inhibits protein synthesis, does not prevent the elongation of cytoplasmic processes in X-irradiated cells. The cytoplasmic extrusions probably result from the assembly of microtubules, since colchicine prevents it. Moreover, ultrastructural examination of these elongated processes shows a significant enrichment in microtubules and microfibrils, as compared to the controls. Cytochalasine B (5ug/ml), a poison of the contractile microfilament machinery, induces like X-rays, the formation of abnormally elongated cytoplasmic processes. But these processes are cytologically and ultrastructurally different from those induced by X-rays : they are enlarged, undulating, with local branching and fuzziness. These cells do not show any increase in the microtubules and microfilaments over the controls. The morphological modifications observed after X-irradiation are stable, since the proportion of cells with long processes increases during four weeks at least. Our results on the effects of X-rays on

glial tumor cells are similar to those previously found in irradiated neuroblastoma cells. The mechanism by which X-rays induce differentiation in neuroblastoma and glial tumor cells still remains to be elucidated.

b. Radiosensitivity of a synkaryon obtained by fusion (V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti).

The X-ray sensitivity of synkaryon 18 has been studied. This synkaryon results from the fusion of two mutants of Chinese Hamster fibroblasts : A23 (deficient in thymidine kinase) and WG 3H (deficient in hypoxanthine-guanosine phosphoribosyl transferase). The survival of the synkaryon and his parental cells has been established by two methods: determination of cell viability during the week following irradiation and determination of cell ability to proliferate and form a macroscopic colony (Puck, 1956). In the days following irradiation, no difference in X-ray sensitivity has been observed between the synkaryon and the parental cells. Preliminary results obtained by Puck's method show a greater sensitivity of the synkaryon (DO=190R) as compared to the A23 strain (DO=220R) and the WG3H strain (DO=290R).

Publications.

V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti-Bosseler and G. Steinert. Morphological modifications of rat glial tumor cells after X-irradiation. Radiation Research, in press.

2 Communications have been presented by V. Heilporn, A. Lievens, S. Limbosch, F. Zampetti at the :

1. Joint meeting of belgian Society of Radiobiologists and Netherlands Radiobiological Society" Rijswijk, May 1972
2. Table ronde sur les cultures cellulaires. Strasbourg (France) November 1972.

Résultats du projet n° V

Chef du projet et collaborateurs scientifiques :

R. Jeener, M. Wikler, G. Urbain-Vansanten, J. Urbain, C. De Vos-Cloetens, A. Van Acker, D. De Sutter, A. Vienne, C. Biéva, N. Tasiaux, N. Laurent, C. Bruyns, R. Leuwenkroon, H. Balluet.

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.

Description des résultats

- The diversity of immunoglobulins that an animal can synthesize during its lifetime corresponds to the presence of an enormous library of clones. Each clone seems to be precommitted to the synthesis of one molecular species of immunoglobulins.

The product of a given clone can be characterized by its isoelectric spectrum, its affinity for the corresponding antigen and by its pattern of idiotypic specificities. Irradiated recipient rabbits grafted with allogeneic lymphoid cells from hyperimmune donors of different genotype have been used to investigate the relationship between memory cells and antibody producing cells.

The antibodies synthesized in the irradiated recipients have a restricted heterogeneity. They possess allotypic specificities of the recipient. Therefore antibodies seem to be synthesized by host radioresistant cells.

However some characteristics of the donor antibody are present on the recipient antibodies. They exhibit a high relative affinity and they react with antiidiotypic sera directed against the individual antigenic specificities of the donor antibody. These results are compatible with an hypothesis involving an information transfer between memory cells and antibody producing cells.

Alternatively the results may be explained by an idiotypic modulation of the repertoire of antibodies synthesized by an animal. (J. Urbain, G. Urbain-Vansanten, A. Van Acker, C. De Vos-Cloetens, N. Tasiaux).

- Irradiated mice (650R) have their immunocompetent system impaired.

As time goes on, there is a restoration of the immunologic potential. This restoration can be inhibited if irradiated mice are grafted with syngeneic cells obtained from a tolerant donor. The tolerant cells seem to be able to synthesize inhibitors of the expression of new clones of the same specificity. (C. De Vos-Cloetens, N. Laurent).

- Irradiated rabbits are injected with spleen cells and lymph nodes from rabbits hyperimmunized with streptococcal vaccine. Oligoclonal anticarbohydrate antibodies produced by the recipients are compared to the donor by isoelectric focusing and by their relative affinities. They appear to be synthesized by the same cellular clones, which moreover possess the same relationship of dominant antibody components. Antibody with new characteristics may appear later during the immune response in the host rabbit. (C. Bruyns, M. Wikler).

- In order to investigate more directly the questions raised by the experiments described above, the isolation of messenger RNA for anti-TMV antibodies has been performed. These messengers have been isolated from polysomes of lymph nodes, taken in rabbit hyperimmunized against TMV. (H. Balluet).

Liste des Publications

J. URBAIN, A. VAN ACKER, C. DE VOS-CLOETENS, G. URBAIN-VANSANTEN : "Increase and decrease of binding affinity of antibodies during the immune response and a possible interpretation". *Immunochemistry*, 1972, 9, 121.

J. URBAIN : "The homologous relationship between the variable and constant parts of immunoglobulins". *Biochemical Genetics*, 1972, 6, 183.

J. URBAIN : "Evolution of immunoglobulins". *Scandinavian Journal of Immunology*, 1972, 1, 3, 292.

G. URBAIN-VANSANTEN, C. DE VOS-CLOETENS, N. TASIAUX, J. URBAIN : "Synthesis of antibodies and immunoglobulins bearing recipient allotypic markers and donor idiotypic specificities in irradiated rabbits grafted with allogeneic hyperimmune cells". (soumis à l'éditeur).

DE SUTTER, D., M. WIKLER : "Mise en évidence d'une tyrosine dans le site actif des anticorps anti-streptocoques homogènes". *Archives Internationales de Physiologie et Biochimie*, 1972, 80, 393.

M. WIKLER : "Comparaison d'anticorps anticarbohydriques de streptocoques et de microcoques produits simultanément par un même animal". *Archives Internationales de Physiologie et Biochimie*, 1973, 81.

M. WIKLER, A. VIENNE : "Relationships between antistreptococcal carbohydrate antibody components". *Scandinavian Journal of Immunology*, 1972, 1, 3, 293.

M. WIKLER, DE SUTTER, D., A. VIENNE : "Structural and functional relationships between streptococcal anticarbohydrate antibodies". 8th FEBS Abstracts, 1972, 684.

C. DE VOS-CLOETENS, N. LAURENT : "Mise en évidence de l'existence de cellules tolérantes capables d'inhiber des cellules normales". *Archives Internationales de Physiologie et Biochimie*, 1973, 81.

J. URBAIN, A. VAN ACKER, N. TASIAUX, C. DE VOS-CLOETENS, G. URBAIN-VANSANTEN : "Interactions cellulaires dans le système immunocompétent et transfert d'information". *Archives Internationales de Physiologie et Biochimie*, 1973, 81.

G. URBAIN-VANSANTEN, A. VAN ACKER, J. URBAIN : "Transfert de cellules lymphoïdes primées à des animaux irradiés". *Archives Internationales de Physiologie et Biochimie*, 1973, 81.

A. VAN ACKER, R. LEUWENKROON, G. URBAIN-VANSANTEN, C. DE VOS-CLOETENS, J. URBAIN : "Evolution des propriétés des anticorps synthétisés au cours d'une immunisation". *Archives Internationales de Physiologie et Biochimie*, 1973, 81.

Chef des Groupes de Recherche :

Dr. Marc A. DALEBROUX, Fonctionnaire Scientifique aux
Communautés Européennes.

Thème général du contrat :

Etude des effets génétiques, aux échelons population et
cellulaire, des rayonnements ionisants :

- I. Effets des radiations ionisantes sur un caractère
de fitness important chez Habrobracon juglandis.

- II. Réactions génétiques cellulaires aux rayonnements
ionisants chez Nicotiana tabacum.

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PROJET N° 1

EFFETS DES RADIATIONS IONISANTES SUR UN CARACTERE DE FITNESS
IMPORTANT (PONTE) CHEZ HABROBRACON JUGLANDIS

Chef du projet : Dr. Marc A. DALEBROUX.

Les cinq expériences à 2000 r sont terminées et ont donné les
principaux résultats ci-après :

(1) Pour les pontes moyennes journalières aussi bien que totales,
la réponse aux génotypes t (témoins), h (hétérozygotes pour les
facteurs mutés) et r (homozygotes pour les facteurs mutés et hété-
rozygotes pour ces facteurs, dans la proportion 1 : 1) est de
type polynomial quadratique dans trois des expériences. Aucune
réponse ne fut décelée dans les deux autres.

(2) Les tests Chi-carrés d'homogénéité des variances intrafamiliales intragénotypes ont été faits. Sur dix tests (pontes moyennes et pontes totales poolées), deux seulement chez les familles témoins sont sous le seuil de signification, alors que tous devraient l'être. Chez les familles h, dont tous les membres d'une même famille sont identiques, sept tests sur dix sont sous le seuil de signification ; ceci est assez conforme à l'espérance. Chez les familles r, on compte quatre tests sous le seuil de signification.

Il apparaît donc que les familles témoins font preuve d'une instabilité considérable. Leur haut degré de consanguinité en est peut-être la cause. Voici, depuis le début de la recherche, les variances intrafamiliales calculées chez les témoins :

Nombre de générations de full-sibbing	F	Variances intrafamiliales témoins	
		sur moyennes	sur totaux
7	0.7344	0.87	35 350
14	0.9398	1.64	57 095
26	0.9953	4.43	107 271
39	0.9997	1.38	48 800
53	1.0000	1.68	88 595
60	1.0000	3.29	144 479
75	1.0000	3.20	139 957
83	1.0000	9.70	328 818

Le rapport est de 1 à 10, ce qui semble beaucoup pour des variances qui devraient être relativement semblables. L'hypothèse d'instabilité de la réponse témoin en fonction de la consanguinité sera vérifiée dans les années à venir.

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PROJET N° II

REACTIONS GENETIQUES CELLULAIRES AUX RAYONNEMENTS CHEZ
NICOTIANA TABACUM

Chef du projet : Dr. Hubert DULIEU, Chargé de Recherche au C.N.R.S.

Monsieur DULIEU a isolé, au Laboratoire de Mutagenèse de l'I.N.R.A. à Dijon, deux loci impliqués dans la différenciation des chloroplastes. A chacun de ces loci, il a obtenu un gène mutant, de sorte que l'on se trouve en présence du système génétique suivant :

$$\begin{array}{ll} a_1^+ \text{ et } a_1 & \text{au locus 1} \\ a_2^+ \text{ et } a_2 & \text{au locus 2} \end{array}$$

Les deux loci sont indépendants en ce qui concerne les fréquences des associations génétiques, mais ils montrent, dans leurs effets conjoints, une forte interaction globale. Les actions géniques seront quantifiées pour chacun des deux loci séparément et pour les deux loci en même temps.

Monsieur DALEBROUX a terminé le travail théorique nécessaire à ces quantifications. Ce travail, trop long pour être développé dans un rapport succinct, sera exposé en détail dans le rapport annuel 1972.

Dans le courant de 1973, deux publications seront consacrées à ce projet.

Associato della Commissione: Università di Pavia - Gruppo Euratom - Istituto di Biologia Generale - Facoltà di Medicina.

N° del contratto: 112-72-1 B101

Capo del gruppo di ricerca: Prof. Marco Fraccaro

Tema generale del contratto: Studi sulla natura, dinamica e modalità di segregazione dei riarrangiamenti cromosomici indotti dalle radiazioni, con l'impiego di tecniche di identificazione cromosomica ad elevato potere diagnostico.

Descrizione generale dei lavori compiuti

Il lavoro si è concentrato su tre linee principali.

- a) Identificazione precisa dei cromosomi implicati in riarrangiamenti strutturali in popolazioni di cellule tumorali umane primarie, non trattate, come modello di anomalie cromosomiche "spontanee" originate in vivo.
- b) Permanenza e destino di specifici segmenti cromosomici identificabili per la loro attività funzionale (eterocromatina costitutiva) in linee stabilizzate in vitro da lungo tempo.
- c) Ricerca di eventuale sensibilità preferenziale alle radiazioni lungo i singoli cromosomi umani.

Descrizione dei risultati

a) Sono state analizzate metafasi da effusioni ascitiche da carcinomi dell'ovaio con le tecniche delle bande Q(fluorescenza) e G(Giemsa). Sono stati identificati in ciascuna cellula i cromosomi normali presenti come tali, mentre numerosi cromosomi che sarebbero stati identificati come normali con le tecniche convenzionali sono risultati in realtà il prodotto di riarrangiamenti strutturali. Tra i cromosomi riarrangiati è stato possibile identificare in molti casi la catena di eventi che li hanno prodotti, rivelando che questi sono delezioni, inversioni e traslocazioni. Certi cromosomi sembrano essere preferenzialmente eliminati (ad esempio i N. 3, 7 ed il cromosoma X), mentre altri lo sono solo apparentemente. Un esempio di quest'ultimo fenomeno riguarda il braccio lungo del cromosoma N. 1 che è risultato implicato in almeno sei differenti riarrangiamenti per traslocazioni che si ripetono in varie cellule entro lo stesso tumore. Nei tumori fino ad ora studiati vi è una relativa costanza del rapporto cromosomi "nuovi"/cromosomi normali entro ciascuna cellula e questo indipendentemente dal numero cromosomico della cellula: questo risultato indica che non sono permesse tutte le combinazioni possibili tra cromosomi normali ed anormali.

Lavori pubblicati

- 1) Fraccaro M.: Fluorescenza differenziale dei cromosomi. Rassegna Clinico-Scientifica, 48, 76, 1972.
 - 2) Fraccaro M.: Fluorescence analysis of somatic chromosomes. Arch. Genetik. 45, 100, 1972.
 - 3) Tiepolo L., Zara C., Zuffardi O., Fraccaro M.: Isochromosome and malignant growth. Lancet, I, 1398, 1972.
 - 4) Tiepolo L., Zuffardi O.: Identification of normal and abnormal chromosomes in tumour cells. Cytogenetics (in press).
- b) I cromosomi della linea cellulare stabilizzata CHEF-125 (origine: embrioni di hamster cinese, Cricetulus griseus, mantenuta in vitro per più di 10 anni) sono stati studiati con le tecniche delle bande Q(fluorescenza),

G(Giemsa) e C(eterocromatina centromerica). Questa linea è pseudodiploide con 22 cromosomi ma vi sono consistentemente due cariotipi con ugual numero cromosomico che differiscono però per un singolo cromosoma. I cromosomi 1, 2, 5, 8 e 9 sono presenti come omologhi normali ed i cromosomi 4, 7 e 11 in singola copia, per un totale di 13 cromosomi normali su 22 in totale. Tra i cromosomi "nuovi", cinque non sono identificabili ma altri due cromosomi dimostrano sezioni di eterocromatina costitutiva del tipo che si trova solo nei cromosomi del sesso delle cellule normali di hamster cinese. Di particolare interesse sono le seguenti osservazioni: 1) la relativamente elevata proporzione di cromosomi normali rimasti tali dopo un così lungo periodo di permanenza in vitro. Questo fenomeno può essere correlato con il mantenimento dello stato pseudodiploide, postulando che persistendo una certa proporzione di cromosomi normali vi è un migliore controllo della segregazione alla mitosi e minore probabilità di perdita o guadagno di cromosomi. 2) La ridistribuzione della cromatina caratteristica dei cromosomi del sesso in un cromosoma marcatore che può essere interpretato come il prodotto di una traslocazione tra un braccio lungo di un cromosoma X ed il braccio lungo di un cromosoma Y. Alternativamente questo cromosoma può essere un cromosoma X ma in questo caso dobbiamo postulare che vi è stato un cambiamento nello stato funzionale di un intero braccio cromosomico.

Lavori pubblicati

- 1) Lo Curto F., Scappaticci Susi, Fraccaro M.: Chromosome identification in a Chinese hamster pseudodiploid cell line (CHEF-125). Cytogenetics, 11, 305, 1972.
- c) Ricerche preliminari su colture di sangue umano irradiate con raggi X durante la fase S e durante la fase G₂ hanno rivelato che le rotture sono localizzate preferenzialmente nelle zone scarsamente fluorescenti.

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 Vertrags : 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 Vertrags :

Consequences of Radiation Exposure : Prevention and Treatment
of Pathological Effects

Allgemeine Darstellung der durchgeführten Arbeiten :

The collaborative research program of the present association
contract No. 088 - 72 - 1 BIA C in 1972 is concerned with the
continuation of previous work - outlined in the Euratom Report
1971 (EUR 4830 d-f-i-n-e) - in 3 areas :

1. Evaluation of damage of ionizing radiation to various organs
but mainly to the hematopoietic tissue.
2. Design and perfection of treatment of bone marrow failure
as seen after whole body irradiation.
3. Selected studies on the physiology and pathophysiology of
cell systems relevant to the understanding of radiobiological
processes.

In the area of evaluation of damage of ionizing radiation, the
research group in Ulm performed a detailed analysis in rats on
the comparative effectiveness of tritiated thymidine and water

in relation to x-irradiation. In these studies, the changes in several organsystems such as hemopoietic tissue and ovaries have been used as endpoints.

The major emphasis of the work of the cooperative project of this contract was on the design and perfection of treatment of bone marrow failure.

In man, the research group in Villejuif studied various aspects of treatment of primary or secondary bone marrow failure by bone marrow transfusion in aseptic environments, by leukocyte and platelet transfusion. In this context, the attempt to treat and prevent "secondary disease" was of paramount importance.

The research group in Ulm continued the cooperative study at the European level within the Gnotobiotic Project Group, supported in part by this contract. The goal of this study is to establish the value of bacterial decontamination and the maintenance of a gnotobiotic state in suitable isolation system for the treatment of hemopoietic failure as also seen after radiation exposure.

The studies in monkeys particularly of the research group in Rijswijk concentrated on the attempts to mitigate secondary disease after bone marrow transplantation using stem cells from phenotypically identical, non-related donors. Successful attempts were made to identify a cellular entity apparently endowed with stem cell potentialities.

The research group in Ulm used dogs to investigate the possibility of the treatment of the gastrointestinal and bone marrow syndrome by means of stem cells separated from the circulating blood and stored at ultralow temperatures. The work is in part based on progress made in the tissue-typing between donor and recipient.

All groups carried out relevant research in rodents which concentrates largely on the effect of antibiotic decontamination and its effect on the survival after x-irradiation and allogeneic bone marrow transplantation. It could be shown both in Ulm and in Rijswijk that bacterial decontamination results in a significant decrease in mortality from secondary disease.

The pathophysiological basis of evaluation and treatment of radiation effects has been studied in all laboratories using different models. Important contributions come from leukemia cell

research because of the general significance of cell kinetic data in such deviated systems for the understanding of all system functions also after radiation injury. Important work has been directed to the characterization and regulation of stem cells and their interrelationship, using various stem cell tests (CFU, ACU etc.). The pharmacological properties of various cytotoxic agents have been investigated and contribute greatly to the understanding of cell function in relation to drug metabolism.

The cooperative work of the partners of this contract is promoted and catalysed by the fact that they form the core membership of both the European Late Effects Project Group (EULEP) and the European Organization for Research on Treatment of Cancer (EORTC) with its Research Clubs (i.e. Stem Cell Club) and Project Groups (i.e. Gnotobiotic Group).

Contractant de la Commission: Association Claude-Bernard,
Institut de Cancérologie et d'Immunogénétique, Hôpital
Paul Brousse, I4 & I6, Av. P.V Couturier, 94.800-VILLEJUIF

N° du contrat: 088-72-1 BIAC

Chef du groupe de recherche: Professeur G. MATHE.

Thème général du contrat: Traitement des états pathologiques secondaires à l'irradiation.

Description générale succincte des travaux réalisés.

Nous avons poursuivi l'étude thérapeutique des états pathologiques secondaires à l'irradiation qui sont de deux ordres: les aplasies de la moelle osseuse et les leucémies.

Dans le cas des aplasies, nos travaux ont porté sur l'hospitalisation aseptique, dont nous réalisons le développement "opérationnel" (construction d'un service en gradient microbiologique), les transfusions de globules blancs, et nous avons montré l'efficacité des cellules provenant des donneurs LMC et l'inocuité, pour ces derniers, des leucophorèses. Nous avons enfin travaillé expérimentalement et cliniquement sur la greffe de moelle osseuse, montrant que les quelques greffes obtenues après conditionnement par ALG ne sont pas compliquées de maladie secondaire mais ne sont que transitoires en raison d'un chimérisme lymphocytaire dissocié, progressant dans le contrôle de la GVH chez les radiochimères, grâce à la découverte des chalone lymphocytaires et grâce à l'emploi de l'antigène d'histocompatibilité soluble.

Dans le cas des leucémies, la chimiothérapie s'étant avérée incapable de "tuer la dernière cellule" puisqu'elle obéit à la cinétique de premier ordre, nous avons introduit la notion d'immunothérapie active qui pourra peut-être s'étendre à l'immunoprévention des leucémies.

Nous avons poursuivi nos expériences de recherche "opérationnelle" en prenant comme modèle la leucémie spontanée lymphoïde de la lignée AkR; nous avons poursuivi nos essais cliniques et publié les résultats obtenus par l'immunothérapie sur plus de 100 cas de leucémie aigue lymphoïde. Nous avons enfin porté nos efforts de développement de cette méthode dans deux directions: celle de l'immunostimulation spécifique en tentant de modifier les cellules et les antigènes tumoraux, et celle de l'immunostimulation non spécifique en mettant au point un screening des adjuvants de l'immunité. Ce screening est actuellement le seul qui existe au monde, et il est devenu parfaitement opérationnel.

Résultats du projet N° 1

Chef du projet et collaborateurs scientifiques:
Professeur G. MATHE.

Titre du projet: Traitement des états pathologiques
secondaires à l'irradiation.

Description des résultats.

L'irradiation entraîne deux sortes d'états pathologiques: l'aplasie de la moelle osseuse, complication constante d'une irradiation aiguë et massive, et la leucémie, complication rare mais pouvant survenir aussi bien après une irradiation aiguë qu'après une irradiation chronique.

Nous décrivons successivement les travaux que notre équipe a consacrés à ces deux sujets, particulièrement grâce à ce contrat.

I. Aplasies de la moelle osseuse

Deux types de traitements sont à considérer, le traitement symptomatique et la greffe de moelle osseuse.

1.1 Traitement symptomatique

1.11 Hospitalisation en environnement aseptique

G. MATHE.

G. MATHE a travaillé à l'avant-projet puis au projet de la construction d'un service "en gradient microbiologique" comportant un étage conventionnel, un étage microbiologiquement contrôlé, et un étage aseptique construit sur le modèle de l'unité aseptique isolée dont l'ICIG dispose depuis 10 ans, et dont il a été le premier institut dans le monde à disposer. Ce développement opérationnel de l'hospitalisation aseptique paraît plus important que le développement technique de systèmes limités à d'exceptionnels malades.

1.12 Transfusion de globules blancs

L. SCHWARZENBERG, G. MATHE, P. POUILLART, R. WEINER, J. LACOUR, J. GENIN, M. SCHNEIDER, F. DE VASSAL, M. HAYAT, J.L. AMIEL, J.R. SCHLUMBERGER, C. JASMIN, et C. ROSENFELD. Chemotherapy with hydroxyurea leucopheresis and splenectomy in the treatment of CML at the problastic phase. Brit. Med. J., 1973, (sous presse).

Les transfusions, aux patients atteints d'aplasies compliquées de septicémie, de globules blancs, de donneurs atteints

de leucémie myéloïde chronique (LMC) sont pratiquées dans le monde entier, et chacun sait qu'elles sont beaucoup plus efficaces que les transfusions de granulocytes prélevés chez les donneurs normaux grâce au séparateur IBM. La question était posée de savoir si les leucophérèses chez les sujets traités obligatoirement par l'hydroxyurée ne réalisaient pas un traitement de la LMC moins efficace quant à la survie que le classique myleran.

L'équipe de l'ICIG a montré qu'il n'en est rien. Il semble même qu'il soit un traitement moins dangereux.

1.2 Greffe de moelle osseuse

1.21 Etudes expérimentales

1.211 Rôle de l'agent conditionnant le receveur d'une greffe de cellules hématopoïétiques et lymphoïdes en tant que facteur favorisant l'insuffisance immunitaire induite par la réaction du greffon contre l'hôte.

M. Gutierrez-Romero et G. Mathé (Transplantation, 1972, 13, 1).

M. Gutierrez-Romero et G. Mathé ont étudié expérimentalement l'influence qu'exerce le procédé de conditionnement du receveur selon qu'il s'agit d'une irradiation totale, de cyclophosphamide ou de SAL, et selon la dose, dans l'insuffisance immunitaire induite par une réaction du greffon contre l'hôte. Ils ont montré qu'elle est essentielle: les fortes doses d'irradiation ou de cyclophosphamide rendent l'insuffisance immunitaire induite par la réaction du greffon contre l'hôte maximale et irréversible. Au contraire, le SAL ne l'affecte que très peu.

1.212 Essai de contrôle de la GVH par les "chalones" lymphocytaires.

Nouvelles données sur le mécanisme et le contrôle du syndrome secondaire.

II Contrôle de la GVH par des inhibiteurs lymphocytaires (chalone?) et par les antigènes d'histocompatibilité.

G. Mathé, E. Garcia-Giralt, N. Kiger, I. Florentin, O. Halle-Pannenko et M.C. Martyré. (Exp. Hematology 1972, 22, 53).

Inhibition de la réaction du greffon contre l'hôte (GVH) par incubation in vitro des lymphocytes du donneur avec les chalones spléniques et thymiques. N. Kiger, I. Florentin, E. Garcia-Giralt, G. Mathé. (Exp. Hematology, 1972, sous presse).

Deux nouveaux moyens de contrôle du syndrome secondaire compliquant la transplantation de moelle osseuse: chalones lymphocytaires et antigènes solubles de transplantation.
G. Mathé, N. Kiger, I. Florentin, E. Garcia-Giralt, M-C. Martyré, O. Halle-Pannenko. Transplantation Proc., 1973, sous presse.

Des expériences ont été menées avec deux types de chalones lymphoïdes, l'une extraite du thymus et déjà mentionnée, l'autre de la rate. On a tenté de prévenir la réaction du greffon contre l'hôte (GVH) par ces deux extraits.

Deux modèles de GVH ont été utilisés: la forme aigue, qui est induite par l'injection veineuse d'au moins 10^7 cellules lymphoïdes, ganglionnaires ou spléniques, d'une des lignées parentales à des hybrides F₁ irradiées sublétalement à la DL 50/30; et une forme chronique, que l'on a établie pour servir de modèle préclinique aussi proche des conditions humaines que possible: les animaux sont irradiés à dose létale et reçoivent 10^7 cellules de moelle osseuse et 10^5 cellules sanguines du donneur. La restauration à 30 jours est excellente, et la mortalité par maladie secondaire est de 100% ou presque; elle s'étale du 30ème au 100ème jour.

Dans ces systèmes, on a mesuré l'intensité de la GVH par différents critères, à savoir: a) la mortalité des receveurs, laquelle est liée à la prolifération des cellules du donneur, qui agressent les tissus de l'hôte; b) la splénomégalie, c) et l'augmentation des ganglions, ces deux derniers phénomènes étant dus à la prolifération mixte des cellules de l'hôte et des cellules du donneur.

Les deux chalones thymique et splénique se sont révélées efficaces dans la prévention de la GVH lorsque l'on a traité: a) le donneur de cellules lymphoïdes, b) le receveur de la greffe, c) les cellules lymphoïdes, par incubation in vitro avec la chalone avant la greffe (1 h à 37°, à raison de 120 gammas de chalone thymique pour 10^7 cellules nucléées et par ml de milieu). Ce dernier traitement semble le plus intéressant au point de vue de ses éventuelles applications cliniques.

On a également vérifié que ce traitement in vitro n'affecte pas la capacité de prolifération des cellules souches de la moelle osseuse ou de la rate: elle ne diminue pas leur

possibilité de former des colonies dans la rate (CFU).

1,213 Essai de contrôle de la GVH par l'antigène d'histocompatibilité soluble.

Nouvelles données sur le mécanisme et le contrôle du syndrome secondaire.

II Contrôle de la GVH par des inhibiteurs lymphocytaires (chalone?) et par les antigènes d'histocompatibilité H-2.

G. Mathé, E. Garcia-Giralt, N. Kiger, I. Florentin, O. Halle-Pannenko, M-C. Martyré. (Exp. Hematology 1972, 22, 53).

Nouvelles données sur la prévention de la GVH: 1) Greffe après conditionnement avec ALG suivie de chimérisme lymphocytaire, 2) Utilisation de chalone lymphocytaires, 3) Utilisation d'antigènes d'histocompatibilité solubles.

G. Mathé, N. Kiger, I. Florentin, E. Garcia-Giralt, M-C. Martyré, O. Halle-Pannenko. (Transplant. Proc. 1972, sous presse).

La maladie secondaire a été induite par la greffe de la moelle osseuse parentale à des receveurs F1 irradiés létalement. La mortalité due à la maladie secondaire a pu être réduite et retardée par l'administration d'extraits comportant des antigènes H-2 solubles obtenus à partir des cellules de foie d'animaux de la lignée des receveurs: soit administration aux donneurs de faibles doses, soit administration aux receveurs eux-mêmes de fortes doses.

1,22 Essais cliniques

1,221 Transplantation de moelle osseuse chez des patients aplasiques, après conditionnement par la globuline anti-lymphocyte (GAL). Absence de maladie secondaire. Chimérisme hématopoïétique dissocié.

L. Schwarzenberg, G. Mathé, J.L. Amiel, M. Schneider, D. Belpomme, J. Choay, P. Trolard, C. Jasmin, C. Rosenfeld, M. Hayat, F. De Vassal et M. Steresco. (Exp. Hematol., 1972, 22, 109)

Transplantation de moelle osseuse après conditionnement par la globuline anti-lymphocytes. Chimérisme hématopoïétique dissocié.

G. Mathé, L. Schwarzenberg, J.L. Amiel, M. Schneider, D. Belpomme, C. Jasmin, C. Rosenfeld, M. Hayat, F. De Vassal et M. Steresco. (Transplant. Proc., 1972, sous presse)

39 patients porteurs d'aplasie médullaire ont été soumis à une transplantation de moelle osseuse après conditionnement par la globuline anti-lymphocytes. Cette transplantation

a été suivie, dans 12 cas, d'une restauration hématologique, et dans dix cas, il a été possible de prouver la prise de la greffe. Aucune maladie secondaire n'a compliqué ces transplantations, et l'explication en réside peut-être dans un chimérisme hématopoïétique dissocié. Chez un malade, on a pu montrer que les immunoglobulines, donc les B-lymphocytes, appartenaient au type du donneur, alors que les T lymphocytes appartenaient au type du receveur. Ceci explique peut être le caractère transitoire de telles greffes, dont la plus longue ne s'est prolongée que pendant 20 mois. Cependant, la durée cumulative de survie de patients porteurs de telles greffes transitoires est significativement plus longue que celle des patients chez lesquels la transplantation n'a pu être suivie de greffe.

1,222 Greffe de moelle osseuse après conditionnement par la combinaison de G.A.L. et de cyclophosphamide.
G. Mathé, L. Schwarzenberg, J.L. Amiel, M. Schneider,
D. Belpomme, C. Jasmin, C. Rosenfeld, M. Hayat,
F. de Vassal et M. Steresco. (en préparation)

Une transplantation de moelle osseuse après conditionnement par la combinaison de globuline anti-lymphocyte et de cyclophosphamide a été tentée chez deux sortes de malades, a) des patients aplasiques chez lesquels une première transplantation, après conditionnement par la GAL seule, avait échoué; b) des patients leucémiques.

12 malades ont donc subi le conditionnement suivant: injection intra-veineuse du "buffy-coat" du donneur, suivie, le lendemain, et pendant 4 jours, de l'administration de cyclophosphamide (45 mg par kilo et par jour pendant 4 jours), et de l'administration conjointe de globuline anti-lymphocyte pendant 7 à 10 jours, selon la tolérance: 3 patients aplasiques, chez lesquels une première transplantation par la globuline anti-lymphocyte seule avait échoué, ne présentèrent aucun signe de restauration; sur 7 patients porteurs de leucémie aigue myéloïde, 3 furent greffés, et l'un d'eux présenta une maladie secondaire suraigue mortelle; sur 2 patients porteurs de leucémie aigue lymphoïde, 1 fut greffé et présenta une maladie secondaire suraigue mortelle.

2 Traitement des leucémies

2.1 Etudes expérimentales

2,11 Application de l'immunothérapie au traitement des leucémies expérimentales.

Immunothérapie de la leucémie spontanée des souris AkR.

G. Mathé, O. Halle-Pannenko et C. Bourut. Europ. J. Clin. Biol. Res., 1972, I7, 997.

G. Mathé et O. Halle-Pannenko ont abordé la leucémie spontanée AkR dès qu'ils ont pu montrer, avec J.F. Doré, que les souris AkR ne présentent pas de tolérance immunitaire à l'égard des antigènes tumoraux de cette leucémie que l'on sait induite par le virus de Gross. Dans une expérience qui vient d'être terminée, ils ont soumis à un protocole d'immunothérapie identique à celui qui est le plus efficace sur leurs autres modèles (combinaisons de cellules allogéniques portant les mêmes antigènes tumoraux et BCG), des souris AkR âgées de 6 mois (période à laquelle 40% seulement présentent une leucémie décelable). D'autres groupes sont soumis, au cours de la même expérience, soit à une chimiothérapie selon un protocole de Skipper, soit à une combinaison séquentielle de chimiothérapie et d'immunothérapie active (IA). Les résultats de l'expérience montrent un effet significatif de l'IA, qui guérit 30% de l'ensemble des animaux, tandis que la chimiothérapie n'agit pas significativement. Plus important encore, ils laissent voir qu'une chimiothérapie intensive préalable à l'immunothérapie active détériore l'effet de cette dernière.

2,12 Développement des moyens de l'immunothérapie active spécifique.

Immunothérapie de la leucémie spontanée des souris AkR.

G. Mathé, O. Halle-Pannenko et C. Bourut. Europ. J. Clin. Biol. Res., 1972, I7, 997.

L'activité in vivo des extraits solubles à partir d'une leucémie RC 19. Effet de la méthode d'extraction. M.C Martyré, O. Halle-Pannenko et R. Weiner. in Investigation and stimulation of immunity in cancer patients. éd. G. Mathé et R. Weiner, I vol., Paris, Edelberg, 1973, CNRS, Springer Verlag.

Nouvelles observations sur les différences in vitro entre les leucocytes du sang humain normal et leucémique, avant, pendant et après leur établissement

en tant que lignées cellulaires, in XIV International Congress of Hematology, I vol., Sao Paulo, 1972, International Congress of Hematology, ed. C. Rosenfeld, A.M. Venuat, M. Paintrand, et C. Choquet.

Influence du traitement par la neuraminidase de cellules leucémiques de souris, sur leur antigénicité, in vivo.

M. Barat, L. Mouillot, M.C. Lambault, M. Ilbery et R. Motta, (en préparation).

Essai d'immunothérapie d'une leucémie murine transplantable utilisant des cellules traitées par des enzymes.

J.F. Doré, M.J. Hadjiyannakis, A. Coudert, C. Guibout, L. Marholev et K. Imai. Lancet, sous presse.

Les premières expériences avaient montré la nécessité, dans l'emploi de l'immunomanipulation pour la thérapeutique, de la combinaison de l'immunothérapie spécifique, stimulation par les antigènes tumoraux ou les matériaux les portant, et de l'immunothérapie non spécifique, stimulation par les adjuvants de l'immunité. Les efforts des équipes de l'I.C.I.G. portent sur le développement de ces deux types d'immunostimulation.

L'immunostimulation spécifique est, jusqu'ici réalisée par l'injection ID et SC de cellules tumorales allogéniques irradiées in vitro.

Une des directions de recherche réside dans la tentative de rendre ces cellules plus antigéniques ou plus immunogéniques en intervenant sur leur glycocalix.

G. Mathé et O. Halle-Pannenko ont traité les cellules par la neuraminidase dans leur expérience concernant la leucémie spontanée AkR. Leur résultat est décevant: ils ont enregistré un effet d'immunothérapie légèrement inférieur à celui exercé par les cellules tumorales irradiées et non traitées par cette enzyme.

C. Rosenfeld et A.M Venuat étudient les cellules des lignées permanentes obtenues à partir du sang des patients leucémiques: l'objet est de rechercher si, une fois la lignée établie, les cellules qu'elle produit, et qui peuvent être produites en masse, sont encore leucémiques, en tous cas sont porteuses des antigènes tumoraux et partant, peuvent être utilisées pour l'immunothérapie active des patients. Les premières observations sont encourageantes mais non affirmatives.

Aussi est-on encore condamnés à employer, pour l'immunothérapie active, des cellules prélevées chez les patients qui en portent un nombre suffisant dans le sang au moment d'une phase perceptible (initiale ou rechute) de leur maladie et de les conserver aux très basses températures. Un effort a été entrepris par R. Weiner, R. Oldham, L. Schwarzenberg et O. Pradet-Balade, pour élever le rendement de la conservation de l'antigénicité des cellules conservées.

Celles-ci sont prélevées chez le patient soit à l'aide du séparateur in vivo à débit continu IBM, soit par sédimentation en présence de substances macromoléculaires comme le dextran; les cellules leucémiques sont ensuite conservées dans du diméthyl sulfoxyde à 10%, après une descente frigorifique régulière de 1°C/minute jusqu'à -30° (appareil de Begg-Bowles), puis conservées à -196° (azote liquide): des ampoules de 10^8 cellules sont ainsi conservées, prêtes à l'emploi immédiatement, après des congélations rapides à 40°C.

Barat et coll. ont étudié cinq leucémies induites par le 7-12 diméthyl-benz-(a)-anthracène chez des souris hybrides F1 (C57Bl/6/DBA/2). On a choisi ces leucémies, car des expériences antérieures avaient permis de démontrer, pour les cinq, qu'une préinjection de cellules leucémiques tuées ne permet pas aux animaux F1 de réagir davantage contre une deuxième inoculation de la même leucémie. Ces cinq leucémies possèdent donc la propriété d'avoir des antigènes leucémiques spécifiques (car il est possible d'obtenir des antisera cytotoxiques spécifiques contre chaque leucémie chez d'autres animaux que les F1), mais de ne pas susciter de réaction immunitaire efficace, in vivo, chez l'hôte syngénique. Dans cette situation, il a paru intéressant aux auteurs d'étudier l'action de la neuraminidase, qui est connue pour rendre les antigènes tumoraux plus immunogènes pour l'hôte.

Des groupes de souris F1 ont été traités par des injections uniques ou répétées de cellules leucémiques, traitées ou non par la neuraminidase, puis irradiées. Dix jours plus tard, une injection de cellules viables de la même leucémie, en quantité variable, a permis de juger de l'influence

du traitement par la neuraminidase dans le cas de chaque leucémie. Seule, une des leucémies a montré une plus grande immunogénicité après traitement des cellules par la neuraminidase. Dans un deuxième cas, la neuraminidase semble avoir accéléré le développement de la leucémie (facilitation?). Dans les trois autres, aucun effet n'a été observé. Ces observations montrent que l'effet du traitement par la neuraminidase dépend des propriétés particulières de chaque leucémie, et qu'un résultat obtenu dans un système expérimental ne peut être extrapolé dans un autre.

L'augmentation d'immunogénicité est reproductible mais de faible intensité. Ce peu d'efficacité du traitement par la neuraminidase est peut être à mettre sur le compte de la faible antigénicité potentielle des leucémies étudiées. On peut rappeler ici que la plupart des résultats publiés sur l'effet du traitement par la neuraminidase concernent des tumeurs ou des leucémies ayant perdu leur spécificité d'hôte, mais cependant génétiquement différentes de l'animal porteur.

Le traitement de cellules tumorales par des enzymes peut augmenter leur pouvoir immunogène (neuraminidase) ou le nombre de leurs déterminants antigéniques (papaïne), (Coudert et coll, C.R. Acad. Sci., 1972, 274, 2833). Cependant, Doré et coll ont conduit un essai d'immunothérapie d'une leucémie murine transplantable (leucémie E (AkR)), induite chez des souris C57Bl/6 par le virus de Gross, basée sur l'injection de cellules traitées par la neuraminidase ou la papaïne et qui abolit l'effet attendu de l'immunothérapie active spécifique.

Des souris C57Bl/6, randomisées en quatre groupes, ont reçu au jour 0 une injection intrapéritonéale de 10^3 cellules leucémiques isogéniques E (AkR). Les souris du premier groupe n'ont pas reçu de traitement. Les souris du second groupe ont reçu, aux jours 1, 5, 9, 13 et 17, 5 injections sous-cutanées de 5×10^6 cellules E (AkR) irradiées. Les souris des troisième et quatrième groupes ont reçu, aux mêmes jours, cinq injections s.c du même nombre de cellules E (AkR) irradiées, traitées soit par la neuraminidase du vibrio choléra (2 unités pour 5×10^6 cellules, 20 minutes à 37°C), soit par la papaïne (0,1 mg/ml d'extrait brut de Papaya pour 10^7 cellules, 10 minutes à 25°C). L'effet modéré de l'immunothérapie active

spécifique est aboli lorsque les cellules utilisées ont été traitées par la neuraminidase ou la papaïne, bien que les cellules lymphoïdes des souris ainsi traitées soient capables "in vitro" de tuer les cellules E (AkR) plus activement que les cellules lymphoïdes des souris des deux premiers groupes. En outre, des anticorps humoraux ont été mis en évidence dans le sérum des animaux du troisième et quatrième groupe.

Ces résultats, qui ne contredisent pas nécessairement les résultats obtenus par d'autres auteurs pour d'autres tumeurs, illustrent la nécessité d'une expérimentation animale avant d'utiliser un traitement enzymatique des cellules utilisées pour l'immunothérapie des leucémies humaines.

2.13 Développement des moyens de l'immunothérapie active non spécifique: pharmacologie des adjuvants systémiques de l'immunité.

A été essentiellement utilisée jusqu'ici, en immunothérapie clinique, l'application de BCG sur des scarifications. Les efforts portent, dans ce domaine de l'immunomanipulation non spécifique, à tenter de trouver des adjuvants systémiques de l'immunité, dont l'application puisse être plus quantifiable et plus confortable pour les patients.

2.131 Préparation et caractérisation de nouveaux adjuvants.

L'activité adjuvante et le pouvoir d'induire une hypersensibilité spécifique du BCG.

I.J. Hiu et J.L. Amiel (Experientia, 1972, 28, 953)

Le résidu bacillaire délipidé du BCG, obtenu par extraction exhaustive du bacille, successivement avec de l'eau distillée puis avec des solvants organiques neutres, est traité par l'anhydride acétique dans la pyridine anhydre. Le BCG ainsi traité perd, à poids égal, par rapport au bacille vivant, une partie de son pouvoir d'induire une hypersensibilité retardée spécifique, mais il accroît son pouvoir de stimulation non spécifique des réactions immunitaires.

Adjuvantivité et teneur en acides animés.

I.J. Hiu, (non publié).

Le "pelage" de la paroi du BCG par traitement acide suivi d'extraction avec des solvants organiques neutres, permet l'isolement de trois fractions chimiquement bien définies appelées successivement "fraction A, B, et C". Les fractions A et B sont des macromolécules peptidoglycolipidiques

contenant les acides animés caractéristiques des cires D de souches humaines de *Mycobacterium tuberculosis*. La fraction B se distingue de la fraction A par sa faible teneur en acides animés. La fraction A est active comme adjuvant de l'immunité, alors que la fraction B ne possède pas d'activité adjuvante. L'absence de l'activité adjuvante de la fraction B apparaît comme liée à sa faible teneur en acides animés formant la partie peptidique de la molécule.

Site responsable de l'activité adjuvante des cires D, peptidoglycolipides isolés de *M. tuberculosis*.
I.J. Hiu (non publié).

L'étude comparative des dérivés des cires D que Hiu a préparés, permet la localisation de groupements responsables de l'activité adjuvante des cires D.

Le déblocage par action ménagée de la potasse, des groupements hydroxylés de la fraction mucopolysaccharidique des cires D de souches humaines de *Mycobacterium tuberculosis*, permet d'obtenir un nouveau dérivé de faible activité adjuvante.

Adjuvant hydrosoluble isolé du BCG.
I.J. Hiu (*Nature New Biology*, 1972, 238, 241)

Le traitement par l'hydrogène dans l'éthanol contenant de l'acide acétique, du résidu bacillaire délipidé du BCG, permet la libération d'une fraction à activité adjuvante appelée MAAF. Les analyses physicochimiques du MAAF montrent qu'il s'agit d'une fraction contenant principalement de l'arabinose et du galactose dans un rapport molaire approximatif de 1 à 2.

Le MAAF s'est révélé actif comme adjuvant des réactions immunitaires à médiation cellulaire (hypersensibilité retardée chez le Cobaye) et humorale (formation d'anticorps anti-globules rouges de mouton chez la Souris).

2,132 "Screening" expérimental

Un "screening" expérimental pour les adjuvants systémiques de l'immunité applicable à l'immunothérapie des cancers.

C. Mathé, M. Kamel, M. Dezfulian, O. Halle-Pannenko, et C. Bourut.

in Investigation and stimulation of immunity in cancer patients, éd. G. Mathé et R. Weiner, I vol., Paris, Edelberg 1973, CNRS, Springer Verlag.

BCG dans l'immunothérapie des cancers. I) Essais expérimentaux et cliniques de son emploi sur le traitement de la maladie résiduelle laissée par la chimiothérapie dans la leucémie lymphoïde aigue. G. Mathé, R. Weiner, P. Pouillart, L. Schwarzenberg, C. Jasmin, M. Schneider, M. Hayat, F. de Vassal et C. Rosenfeld, J.N.C.I., 1973, sous presse.

BCG dans l'immunothérapie des cancers. II) Résultats obtenus avec diverses préparations de BCG dans le "screening" des adjuvants systémiques de l'immunité appliqués à l'immunoprévention ou l'immunothérapie des cancers.

G. Mathé, O. Halle-Pannenko et C. Bourut. J.N.C.I., 1973, sous presse.

G. Mathé a établi une batterie de tests pour soumettre au "screening" les agents susceptibles de se comporter comme adjuvants systémiques de l'immunité actifs dans l'immunoprévention et l'immunothérapie des cancers.

La liste des tests est donnée au tableau 1 et la liste des substances qui leur ont été soumis dans les deux précédentes années est mentionnée au tableau 2.

Ce travail a montré que des agents considérés, en raison d'expériences antérieures, comme des adjuvants de l'immunité, sont réellement, dans certaines conditions, des immunostimulants, mais qu'ils peuvent être, dans d'autres conditions, des immunodépresseurs, et cela non seulement dans le test de Jerne (T & B dépendants), mais dans celui de la GVH (T-dépendant). Il a été observé que, tandis que leur effet est, soit nul soit favorable en ce qui concerne l'immunoprévention de la leucémie L 1210, il peut être nul, favorable ou défavorable (par effet de facilitation immunitaire) dans le cas des deux tumeurs solides, la tumeur de Lewis et la tumeur ICIG C₁.

Ce travail a été prolongé dans deux directions, respectivement pratique et théorique.

La première concerne la comparaison, à l'aide de cette batterie de tests, de dix préparations de BCG, le BCG frais de l'Institut Pasteur (I.P.) s'étant révélé le plus efficace des dix agents testés plus haut. On a donc comparé, dans une seconde étude, l'effet de cette préparation fraîche de BCG de l'IP à neuf autres préparations de BCG, toutes lyophilisées mais provenant de différentes sources. La première s'est révélée, de très loin, la plus efficace.

La seconde direction de cette étude expérimentale des adjuvants, consiste à les soumettre à une série de tests capables de révéler leurs actions respectives sur l'immunité à médiation cellulaire et sur l'immunité à médiation humorale. Ce travail coopérateur est en cours.

TABLEAU I

Tests et méthodes utilisés pour le screening

Techniques	Jour 0	Injection de l'adjuvant		Résultats
		Avant°	Après°	
TEST DES PLAQUES D'HEMOLYSE (JERNE)	10^9 GLOBULES ROUGES DE MOUTON, I.P.	JOURS -14, ou -5, ou - 2,5	JOURS 0, ou + 1 ou + 2, ou + 3	PFC au jour + 4
G.V.H.	10^7 CELLULES MEDUL- LAIRES et $2,5 \cdot 10^7$ CELLULES GANGLION- NAIRES C57Bl/6 CHEZ DES SOURIS F1 (DBA/2 x C57Bl/6)	JOUR - 14 (BCG) JOUR - 2,5 (autres)	-	DECES
L 1210	10^3 CELLULES LEUCE- MIQUES, I.V.	JOUR - 14 (BCG) JOUR - 2,5 (autres)	-	DECES
TUMEUR DE LEWIS	$2 \cdot 10^6$ CELLULES TU- MORALES, S.C.	JOUR - 14 (BCG) JOUR - 2,5 (autres)	-	DECES
ICIG C ₁	$2 \cdot 10^6$ CELLULES TU- MORALES, S.C.	JOUR - 14 (BCG) JOUR - 2,5 (autres)	-	DECES

° l'administration de l'antigène (test de Jerne), le transfert des lymphocytes (GVH) ou l'inoculation de la tumeur

TABLEAU 2

CANDIDATS-ADJUVANTS SYSTEMIQUES DE L'IMMUNITE SOUMIS A LA PREMIERE PARTIE DU "SCREENING"

AGENTS	SOURCE	COMPOSITION	DOSES PAR SOURIS +
BCG	INSTITUT PASTEUR	SUSPENSION DE BACTERIES VIVANTES	1 mg
MER	ICIG (Méthode de Weiss)	BCG SANS FRACTION LIPIDIQUE EXTRAIT PAR LE METHANOL & L'ACETONE	1 mg
HIU I	ICIG	COMPOSANT DE LA MEMBRANE DU BCG (INSOLUBLE)	1 mg
HIU II	ICIG	COMPOSANT DE LA MEMBRANE DU BCG (SOLUBLE)	0,5 mg
C. PARVUM	INSTITUT PASTEUR	SUSPENSION DE BACTERIES MORTES	400
C. GRANULOSUM A	INSTITUT PASTEUR	BACILLES TUES	400
C. GRANULOSUM B	INSTITUT PASTEUR	MEMBRANES TRAITÉES PAR LE FORMOL A FROID	100
C. GRANULOSUM C	INSTITUT PASTEUR	MEMBRANES PRÉPARÉES PAR ACTION MECANIQUE	100
POLY I - POLY C	CHOAY	ACIDE POLYINOSINIQUE ACIDE POLYCYTIDYLIQUE	300
POLY A - POLY U	CHOAY	ACIDE POLYADENOSINIQUE ACIDE POLYURIDINILIQUE	150

+ Déterminées par des études préliminaires.

2,2 Etudes cliniques

2,21 Résultats actuariels des essais d'immunothérapie active.

Essais d'immunothérapie dans la leucémie aigue lymphoïde. Influence de divers facteurs sur les résultats. Etude de 100 malades.

G. Mathé, P. Pouillart, L. Schwarzenberg, M. Hayat, J.L. Amiel, M. Schneider, R. Weiner, C. Jasmin, F. de Vassal et A. Cattan.

a) J.N.C.I., 1972, sous presse.

b) Presse Med., 1972, sous presse.

Les essais cliniques de traitement de la leucémie aigue lymphoïde (LAL) par immunothérapie active ont été poursuivis, et un bilan a pu être fait des résultats qui portent aujourd'hui sur plus de 100 malades.

Rappelons qu'il s'agit de l'essai d'éradication de la maladie résiduelle minimale laissée par une chimioradiothérapie cytoréductrice complémentaire à la chimiothérapie inductrice de la rémission.

L'étude du premier essai qui avait, pour la première fois, démontré l'action de l'immunothérapie active chez l'Homme se poursuit, et ses résultats sont inchangés: 7 patients sur les 20 soumis à l'immunothérapie active sont encore en première rémission, donc n'ont pas présenté de rechute, entre 4 et 7 ans après la fin de la chimiothérapie.

Signalons que Powles et Hamilton-Fairley viennent de confirmer, dans un essai portant sur la leucémie aigue myéloïde et au cours duquel ils ont appliqué l'immunothérapie active selon la même modalité, l'efficacité de ce moyen thérapeutique.

L'étude globale de la centaine de malades traités pour LAL confirme que la courbe actuarielle des durées de première rémission, après une descente, se casse vers le 32ème mois, pour prendre l'allure d'un plateau, qui peut être considéré comme la traduction statistique de l'"espérance de guérison": ce plateau concerne 30% de patients de tous âges et 37% des moins de 15 ans. Les courbes des survies présentent ce plateau pour 43% des malades de tous âges et 62% des moins de 15 ans.

Plus intéressantes encore, deux études qui révèlent l'intervention de deux facteurs dans ce pronostic lié à l'effet de l'immunothérapie active: le type cytologique et la modalité de la chimiothérapie préimmunothérapeutique.

Sur les 4 variétés de LAL que nous avons individualisées et dont nous avons parlé plus haut, seuls présentent les courbes de première rémission avec plateau, le type microlymphoblastique (le plateau concerne 51% des patients de tous âges et 62% des moins de 15 ans) et le type prolymphocytaire (57% et 46%). Le type macrolymphoblastique s'est révélé immunothérapie insensible, mais chimiothérapie sensible, puisque si la courbe de la durée de première rémission ne présente pas de plateau, celle de la durée de survie en présente un. Le type prolymphoblastique n'en présente pour aucune des courbes.

Le second facteur qui semble intervenir dans l'efficacité de l'immunothérapie active est la modalité de la chimiothérapie préimmunothérapeutique. La courbe des durées de première rémission prend l'allure d'un plateau pour tous les protocoles dans lesquels nous avons appliqué une chimiothérapie préimmunothérapeutique longue et d'intensité modérée; elle tombe au contraire régulièrement à 0 dans le cas d'un protocole dont la chimiothérapie préimmunothérapeutique fut courte et intensive.

Des essais cliniques d'IAC sont actuellement à l'étude pour le traitement des sarcomes du type conjonctif.

2,22 Développement

2,221 "Screening" clinique des adjuvants de l'immunité

R. Weiner, E. Garcia-Giralt, M. Schneider et
C. Jasmin (en préparation).

Ce screening consiste à étudier les réponses immunitaires non spécifiques de malades soumis à un traitement par un adjuvant immunitaire systémique. Les épreuves sont faites avant et après traitement chez le même malade. Elles sont aussi faites chez des témoins recevant un placebo.

Les tests utilisés sont mentionnés sur le tableau 3.
Le levo-tétramisole est actuellement soumis à cette étude.

- TABLEAU 3 -

Tests utilisés chez l'Homme pour le "screening" des adjuvants systémiques de l'immunité

TYPE DE LYMPHOCYTES IMPLIQUES	TYPE DE REPONSE ETUDIEE	
	PRIMAIRE	SECONDAIRE
T LYMPHOCYTES	IN VIVO	Réponse d'hypersensibilité cutanée retardée: Au dinitrofluorobenzène, au chlorure de picryl.
	IN VITRO	Réponse d'hypersensibilité cutanée retardée à la tuberculine, la candidine et l'antigène ourlien.
B LYMPHOCYTES	IN VITRO	Transformation des lymphocytes en présence de phytohémagglutinine (x). Culture mixte lymphocytaire.
		Transformation des lymphocytes en présence de tuberculine purifiée PPD.
T + B LYMPHOCYTES	IN VIVO	Electrophorèse des protéines sériques. Dosage des immunoglobulines IgA, IgG et IgM. Dosage du facteur rhumatoïde.
	IN VITRO	Numération sanguine des lymphocytes
	IN VITRO	Transformation des lymphocytes en présence de pokeweed (x)

(x) Il s'agit de la réponse des lymphocytes à un mitogène et non à un antigène. La classification en réponse primaire est donc ici artificielle.

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Associato della Commissione :

ISTITUTO DI RICERCHE FARMACOLOGICHE "MARIO NEGRI"

N° del contratto : 088-72-1 BIAC

Capo dei gruppi di ricerca : Prof. S. GARATTINI.

Tema generale del contratto :

Consequences of radiation exposure, prevention
and treatment of pathological effects.

In 1972 our research efforts have been devoted to the following main aspects:

- (a) drug interactions in immunosuppressive treatment,
- (b) comparative activity and metabolism of 6-mercaptopurine and azathioprine;
- (c) characterization of dimethyltriazano imidazole-carboxamide (D.I.C.) as an immunodepressant;
- (d) antilymphocytic serum on corticosterone levels;
- (e) development of an experimental procedure for in vitro evaluation of cytotoxic drugs active through metabolites.

The first series of research on the possible effects of associating to various immunosuppressants, treatment with other drugs frequently employed in the clinic has been completed as regards the investigations with phenobarbital, a representative agent for this important class of drugs. It has been found that concomitant or antecedent treatment with such a compound

can markedly modify in both directions (increase and decrease) the in vivo activity of a number of cytotoxics. It has also been established that the type of treatment (acute or prolonged) with phenobarbital or with the cytotoxic can influence, to a great extent, the in vivo activity of the cytotoxics. As regards point (b), the investigations have been completed showing that in rodents 6-mercaptopurine is more immunosuppressive than azathioprine by parenteral injections while the reverse is true for oral treatments. Studies aimed at elucidating this differential activity have permitted to advance a possible role for the imidazole moiety released in vivo from azathioprine in giving the observed results.

A recently developed radiomimetic, D.I.C., has been investigated and characterized for its immunosuppressive activity in mice. The compound was found to possess very potent activity in interfering with primary and secondary immune responses involving circulating or cell-bound antibodies.

As regards point (d), further investigations on the mechanisms underlying ALS-induced corticosterone levels have been performed showing the specificity of the effect, and that ALS can modify the liver metabolic capacity towards steroids.

The development of a method for the in vitro recognition and assessment of cytotoxic drugs active only through metabolites has been completed and initial results are presented in one of the publications attached hereafter.

Included is also a list of other publications prepared during 1972 in this Institute and supported by EURATOM Contract No. 088-72-1 BIAC.

1. Drug Interactions in Immunosuppressive Treatment

Federico Spreafico, Annunciata Vecchi, Alessandro Anaclerio,
Alberto Mantovani

Two examples of such interactions have been considered, namely (i) the association between immunosuppressants and drugs widely used in the clinic such as derivatives of barbituric acid (specifically phenobarbital), and (ii) the association between two immunosuppressants. As regards point (i), it has been found that phenobarbital, by itself devoid of any activity on the immune response at the doses and schedules employed, can profoundly alter the effect of a number of subsequently or concomitantly administered cytotoxic agents. In fact, short or more prolonged pretreatments with phenobarbital lead to an increase of various degrees in the in vivo activity of subsequently injected doses of 6-mercaptopurine, bis-chloroethylnitrosourea, methylimidazolcarboxamide and procarbazine. The same treatments lead to a marked reduction in the activity of single doses of cyclophosphamide and hydrocortisone whereas other agents such as methotrexate, hydroxyurea, triethylenmelamine, daunomycin, ALS, etc. are unmodified. When instead of single doses of immunosuppressants, more prolonged courses of the drug are given in conjunction with chronic phenobarbital treatment, modifications of opposite type in the immunosuppressive activity can be observed. For instance, the activity of single doses of cyclophosphamide is reduced by short or more sustained courses of phenobarbital whereas greater immunosuppressive activity is obtained when repeated injections of the radiomimetic are employed. The most probable mechanisms underlying these observations are proposed to lie in the "inducing" capacity of phenobarbital on liver microsomal enzymes as indicated also by investigations of the circulating levels of immunosuppressants in these conditions.

A series of parallel investigations have been devoted to study the modifications in antileukemic activity of the same compounds; antitumor activity was found to parallel the modification already described for immunosuppressive potency.

As regards point (ii), experiments performed have revealed that not in every case the association of two immunosuppressants leads to increased in vivo activity, for instance, when the administration of methotrexate is preceded by the injection of L-asparaginase, both cytostatic agents frequently employed in the clinic for their remarkable characteristics, a reduction in immunosuppressive activity is observed. No reduction, neither synergism, is found when asparaginase follows methotrexate injections. A series of other compounds are currently being investigated in order to check whether antagonisms or synergism follow other types of combined therapy.

2. Comparative Activity and Pharmacokinetics of
6-Mercaptopurine and Azathioprine

Federico Spreafico, Maria Grazia Donelli, Adriana Bossi,
Annunciata Vecchi, Silvio Garattini

The comparison of the immunosuppressive activities and pharmacokinetic characteristics of 6-mercaptopurine and azathioprine has been completed. A copy of a paper regarding this work is enclosed. In summary, the investigations performed have shown that contrarily to a commonly held opinion, azathioprine (the drug most widely used in current human immunosuppressive treatment) is not in every condition a stronger immunosuppressant agent than the parent compound 6-mercaptopurine. In fact, the latter is markedly more active (2-3 times) in rodents when injected by parenteral routes of administration whereas azathioprine is more active when given orally. The dose-response curves for both agents appear to best fit an hyperbolic with shoulder type of curves, i.e. the curves tend to straighten with increasing doses. In order to elucidate the possible reason for such differential in vivo activity, studies were undertaken of the levels, in blood and spleen, of 6-mercaptopurine after injection of either compound. It was found that a very rapid splitting (more than 70% in 1 min) of azathioprine to yield 6-mercaptopurine occurs in vivo and that essentially similar 6-mercaptopurine concentrations were found after administration of equimolar doses of both compounds. The possible role of the imidazole moiety released from the in vivo splitting of azathioprine was then considered; results obtained are consistent with the hypothesis that such a moiety plays a role in the reduced in vivo activity of azathioprine. The possible implications of the described findings are discussed in the paper attached. (Attached paper no. 1).

Characterization of the Immunosuppressive Activity of Dimethyltriazeno Imidazol-carboxamide

Federico Spreafico, Alessandro Anacclerio, Alberto Mantovani, Annunciata Vecchi

A recently developed compound, dimethyltriazeno imidazol-carboxamide (NSC 45388) shown to possess powerful antitumor activity in some systems, has been characterized for its immunosuppressive activity, also in the frame of other experimental research lines of interest for this Institute (vide point 3 of our research proposal 1972-1975). This compound was found to have very marked immunosuppressive activity in rodents in terms of reduction of humoral as well as cell-mediated responses after primary or secondary stimulation. The compound is immunosuppressive when injected before, concomitantly with or after antigen administration; in terms of Jerne's plaque inhibition two peaks (4 days before and 2 days after immune challenge, respectively) for the immunosuppressive activity were observed, however the highest activity was seen when the compound was given before stimulation. The state of immune depression appears to be quite long-lasting since even relatively low doses of the drug injected as a single dose 10 days before antigen still give significant degrees of reduction in the PFC response in mice. The dose-response curve appears to best fit an exponential, i.e. the same type of that given by alkylating agents, thus sustaining ideas currently held about the drug's biochemical mechanisms of action. Still in progress are investigations on the possible comparative myelotoxic activity of the compound as well as its inhibitory activity on the in vitro responsiveness to PHA and antigenic stimulation of human lymphocytes, an experimental set up which should also allow to determine whether the compound acts as such or only after metabolic transformation in the body.

4. Antilymphocytic Serum and Adrenal Hormones

Viviana Marc, Federico Spreafico

Antilymphocytic serum (ALS) is known to be an immunosuppressant of remarkable characteristics and is already widely employed in the clinic, however still relatively little is known of many of its pharmacological aspects regarding its use. In most of the cases this agent is employed in conjunction with other immunosuppressive agents, notably corticosteroids. It has been found that when ALS prepared in rabbits is injected in rats or mice, a prompt and marked increase in circulating corticosterone levels can be observed, reaching levels 2-3 times higher than those found in control animals (i.e. injected with saline or normal rabbit serum /NRS/).

Such high levels are of relatively prompt appearance following single or repeated injections and are maintained for a relatively long period of time, since abnormal levels are still found 5 days after a course of 4 injections of ALS. That this effect should be considered specific for ALS and not simply a stress response, seems indicated by the fact that no changes in body or adrenal weights were recorded, that NRS did not cause modifications of corticosterone levels of degrees and durations comparable to those given by ALS, that purified specific antilymphocytic antibodies obtained through a method described by one of us (F.S.) were fully capable of raising corticosterone levels, and, lastly, that ALS absorbed in vitro with lymphocytes was devoid of any activity in this respect. In an effort to pinpoint the possible mechanisms underlying such ALS effect, it was found that dexamethasone pretreatment, a drug known to specifically block ACTH release, was capable of totally preventing the ALS-induced corticosterone increase thus pointing that the mechanism responsible for such an effect may not solely nor mainly involve the adrenals but possibly the hypothalamus-hypophysis axis. The liver metabolic capacity of ALS-treated animals has also been investigated studying the activity in

vitro of liver microsomal preparations. It was found that liver microsomes from ALS-treated animals were capable of metabolizing steroids in significantly greater amounts than preparation obtained from saline or NRS-treated controls. These findings may be of some relevance in elucidating the complex mechanisms underlying the immunosuppressive and anti-inflammatory properties of ALS, at the same time they indicate that the activity of the liver microsomes, a system responsible for the metabolism of many endogenous and exogenous substrates, after ALS treatments even of short duration may be abnormal: an observation that may be of some importance in planning rationalized treatments with ALS in combination with other immunosuppressants.

5. Evaluation in Vitro of Cytotoxic Compounds Active through Metabolites

Ersilia Dolfini, Alfredo Martini, Maria Grazia Donelli,
Luciano Morasca, Silvio Garattini

The work designed to set up a method allowing the evaluation under in vitro conditions of the cytotoxic activity of drugs active through metabolites, already partially presented in a previous report, has been completed. For a full presentation and discussion of the data please refer to the attached paper no.2 In brief, the method was designed to obviate the limitations presented by normal in vitro tests in the case of cytotoxic compounds which do not act per se but only after bodily transformation, a representative model substance being cyclophosphamide that needs to be actively processed by the liver microsomal system before exerting its activity. In the procedure described, therefore, in vitro cultured cells are incubated with the drug and liver microsomal preparations together with necessary cofactors, (i.e. with the **system mainly responsible** for the metabolism of foreign compounds in the intact organism). This method seems to be of some general interest not only for better characterizing agents of potential interests as cytotoxic both in immunosuppressive and antitumor treatments, but it may be also be of value for giving further insight into their mechanisms of action.

Papers Submitted for Publication in 1972

1. Chemotherapeutic 'Fingerprints' of Two Experimental Tumors in Vitro.
L. Morasca, G. Balconi, F. De Nadai, E. Dolfini
Europ. J. Cancer , 8:429-435, 1972.
2. A Spectrophotometric Method for the Estimation of the Carcinostatic Agent 6-Chrysenamine (E.O.R.T.C. 116) in Biological Fluids and Tissues.
G. Franchi, A. Forgione, S. Filippeschi, J. Csetényi, S. Garattini.
Submitted to Europ. J. Cancer, 1972.
3. Specific Problems in Cancer Chemotherapy: Drug Interactions.
S. Garattini, M.G. Donelli, F. Spreafico
Proc. 5th Internat. Congress on Pharmacology, San Francisco, 1972.
4. Distribution of 6-Mercaptopurine in Tumor-bearing Animals.
M.G. Donelli, T. Colombo, A. Forgione, S. Garattini.
Submitted to Biochem. Pharmacol., 1972.
5. Chemotherapy of a Spontaneous Mammary Carcinoma in Mice. Relation between in Vitro - in Vivo Activity, and Blood and Tumor Concentrations of Several Antitumoral Drugs.
G. Balconi, A. Bossi, M.G. Donelli, S. Filippeschi, G. Franchi, L. Morasca, S. Garattini.
Submitted to Cancer Chemoth. Rep., 1972.

Vertragspartner der Kommission :

Land Baden-Württemberg, vertreten durch die Universität Ulm

Nr. des Vertrags : 068-72-1 BIAC

Leiter der Forschungsgruppe : Prof. Dr. Theodor M. Fliedner

Allgemeines Thema des Vertrags :

Effects of ionizing radiation on mammalian organisms
and their treatment

Allgemeine Darstellung der durchgeführten Arbeiten :

The project work of the research group in Ulm concentrated in 1972 on the study of the pathogenesis of radiation injury caused by incorporation of radionuclids such as tritium in the form of tritiated thymidine and tritiated water as compared to external irradiation. The models used were prenatal administration of tritiated compounds or x-irradiation with postnatal measurement of effects. The goal was to establish the quality factor for tritium as compared to x-ray exposure. Another area of work dealt with the development and improvements of methods to treat mammals after supralethal whole body irradiation. The model used is the beagle exposed to 1200 r x-irradiation. The gastrointestinal phase of the radiation syndrome is overcome by systematic treatment with electrolyte solutions and antibiotic therapy. Hemopoietic regeneration is achieved by post-irradiation transfusion of hemopoietic stem cells that are collected among mononuclear leukocytes from the peripheral blood by means of an extracorporeal shunt and an IEM blood cell separator. Basic studies deal with the quantitative and qualitative characterization of such blood stem cells and their regulation, with possibilities to preserve them at ultralow temperatures and with demonstration of their proliferative and differentiation potential by means of the soft-agar colony technique. Both, the radiobiological as well as the therapeutic studies depend largely on the advances made in the field of basic research. The relevant research to-day concerns itself with the functional characterization of hemopoietic stem cell pools. Thus, the Ulm group concentrated on the investigation of the interrelationship between the uncommitted stem cell pool and the pools of cells that are committed to differentiate into certain cell lineages upon specific stimuli such as erythropoietin.

Ergebnisse des Projektes Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter :

W. Schreml with W. Calvo, T.M. Fliedner, R.J. Haas
E.B. Harriss and M. Spoljar

Titel des Projekts :

Investigations on the early and late toxicity of tritium in the mammal, with particular respect to its relative biological effectiveness and its mode of action in comparison with external or internal radiation of other qualities

Darstellung der Ergebnisse :

Two lines of interest were primarily followed in the 1972 studies of the radiotoxic effect of tritium :

1. Relative radiotoxic effectiveness of tritiated thymidine and tritiated water.

2. Dosimetry of incorporated tritium activity.

For the relative radiotoxic effectiveness of $^3\text{H-TdR}$ and HTO in the system of continuous in utero exposure of developing rats from day 9 to term, an effectiveness factor of 10 was found for various parameters, most clearly for the number of oocytes in newborn rats. As seen in Table 1, a ten times higher amount of tritium activity had to be injected to achieve a comparable reduction of oocyte number in the offspring. To interpret this large difference in effectiveness, the distribution and biochemical characteristics of the incorporated activity had to be studied. Figure 1 shows the relative distribution of radioactivity in homogenates of whole animals exposed to $^3\text{H-TdR}$ and HTO . HTO is almost exclusively found in a volatile form. Of the activity in $^3\text{H-TdR}$ animals, approximately 70 % is in a volatile form, while the remaining activity is primarily incorporated into DNA. Calculating the dose delivered by the volatile, homogeneously distributed activity for those groups in which a 50 % reduction of oocyte number occurred, 15.5 rads/day are calculated for the HTO group and 0.98 rads/day for the $^3\text{H-TdR}$ group. The additional dose delivered to the oocyte nuclei by DNA-bound activity in the $^3\text{H-TdR}$ group was calculated from the specific activity of DNA. This dose amounted to 0.64 rads/day. The dose calculations were made on ovaries of newborn animals. It is clear that the dose calculation on the level of the oocyte nucleus does not explain

the difference in a radiotoxic effects between the two tritium labelled molecules on a distributional basis. Further studies are under way to explain the difference in radiotoxic effectiveness.

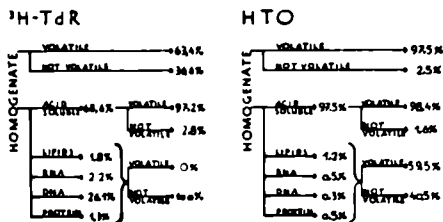


Fig. 1

Relative distribution of radioactivity in homogenates of whole animals exposed to $^3\text{H-TdR}$ and HTO. The upper part indicates the results of lyophilisation. In the lower part, the homogenates are stepwise extracted and the individual fractions subjected to lyophilisation.

Reduction of Total Oocyte Number %	uCi / day Given to Mothers	
	HTO	$^3\text{H-TdR}$
0	290	
30		72
54	1440	
56		144
96	2900	
99		290

Fig. 2

Reduction of total oocyte number in % of normal by different doses of $^3\text{H-TdR}$ and HTO, infused into pregnant rats from day 9 to day 22 of pregnancy. The oocyte number was determined in the newborn animals.

Ergebnisse des Projekts Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter :

W. Calvo with T.M. Fliedner, J. Forteza-Vila, R.J. Haas and E.B. Harriss

Titel des Projekts :

Investigations on the pathogenesis of ^3H -thymidine toxicity in mammals following single, repeated or continuous doses and the mechanisms of regeneration

Darstellung der Ergebnisse :

The morphological changes produced in different organs of adult rats by a continuous infusion of ^3H -thymidine were studied. Four-month-old female Wistar rats were injected with a dose of 864 μCi / day using a Harvard pump. From each animal fragments of spleen, ovary, kidney, intestine, tibia, liver and lymph nodes were prepared for morphological and autoradiographic studies.

The presence of a marrow aplasia in some of the animals has already been mentioned (Annual Report 1971, EUR 4830, Vol. II). As a continuation of these studies we centred our interest in the fine structure of the marrow stroma.

The walls of the sinusoids (sinus recti and sinus contorti) showed discontinuities and different degrees of damage within 3 days of beginning the experiment. The cytoplasm of the endothelial cells appeared vacuolized and their mitochondria swollen. Erythrocytes and platelets accumulated in the parenchyma and initiated the formation of thrombi. The walls of the central sinus and these of arterioles (nutrient vessels) did not show changes up to the end of the experiment (18 days of continuous infusion). The nerves of the marrow had minor morphological changes, consisting of accumulation of glycogen granules in nerve-endings and in non-myelinated axons. The nerve trunks showed also an increase in the number of connective tissue fibers (fibrosis) between the 12th and the 18th day of infusion.

The blood forming cells had signs of degeneration such as karyorrhexis, pyknosis and vacuolization of cytoplasm and nuclei. There were also cells showing asynchrony in the differentiation of cytoplasm and nucleus.

The evaluation of the effects of continuous infusion of ^3H -thymidine in other organs is being carried out at the present.

Ergebnisse des Projekts Nr. 3

Leiter des Projekts und wissenschaftliche Mitarbeiter :

B. Kubanek with O. Bock, E. Bock, E.B. Harriss and W. Heit

Titel des Projekts :

Comparative investigations on the damage and repair of hemopoietic cells by ^3H -thymidine or radiomimetic substances, with particular reference to the uncommitted and committed stem cells

Darstellung der Ergebnisse :

^3H -thymidine, radiomimetic and cell-cycle specific substances are widely used to analyse the kinetics of the hemopoietic stem cell pools. Comparative investigations of the in vivo effects of individual substances are, however, meagre and also partly contradictory.

Since Hydroxyurea (HU) and tritiated thymidine (^3H -TdR) seem to be the only useful agents for killing cells in S-phase, we investigated their influence on the different hemopoietic stem cell compartments. Single lethal doses of HU or ^3H -TdR gave a similar initial reduction for the pluripotent stem cells measured by the spleen colony assay (CFU) and also for the committed myeloid stem cell compartment measured by the agar colony forming assay (ACU). However, marked differences were observed when the effect of these agents was investigated on the erythroid committed stem cells (ERC) measured by the response to a standard dose of EP, ^3H -TdR giving a much higher reduction than Hydroxyurea. These differences seem to be due to the fact that in the case of the CFU and ACU measurement samples are taken from the animal at a certain time after the administration of HU or ^3H -TdR and are then tested in lethally irradiated recipient mice or on soft agar plates. Whereas in the case of ERC the response to erythropoietin is measured in the animals which had received the damaging agents. Differences in repair mechanisms, recruitment due to the relatively long half life of EP and further damage due to reutilization of ^3H -TdR may influence the erythropoietic response in this assay where the endpoint is measured some days after the administration of the agents. Therefore, all three stem cell compartments were

investigated up to 5 days after the administration of a single dose of 0.8 mCi ^3H -TdR or 1000 mg/kg HU. Not only an initial difference in the reduction of the ERC compartment was found but also marked differences in the recovery of the three stem cell compartments, as can be seen in Fig. 1. From these results it can be concluded that repopulation of the committed compartments has priority over the restoration of the pluripotent compartment. The marked differences found between both agents are probably at present best explained by a prolonged cell death owing to reutilization of ^3H -labelled DNA breakdown products from initially heavily labelled cells. Further experiments are planned to elucidate the above mentioned hypothesis.

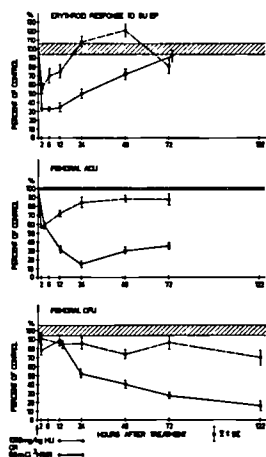


Fig. 1

The influence of 1000 mg/kg Hydroxyurea o - - - o
 or 0.8 mCi ^3H -TdR o — o on the hemopoietic stem
 cells in the mouse

Ergebnisse des Projekts Nr. 4

Leiter des Projekts und wissenschaftliche Mitarbeiter :

T.M. Fliedner with Ch. Bruch, W. Calvo, E.B. Harriss, E. Herbst,
E. Hügl, P. Kovács, H.P. Schnappauf

Titel des Projekts :

Investigations on the possibilities of isolating hemopoietic stem cells from the peripheral blood in dogs (autologous)

Darstellung der Ergebnisse :

In 1972, it has been the purpose of the project studies to demonstrate the feasibility to remove a sufficient number of leukocytes from the circulating blood of dogs (beagles), to repopulate a radiation induced relationship between the number of mononuclear leukocytes transfused and the degree of hemopoietic recovery and to study the conditions for preservation of these cells at ultralow temperatures. Preliminary data have been published in the 1971 Annual EURATOM Report (EUR 4830 d-f-1-n-e). In a control group of 11 dogs, there was no repopulation of any bone marrow sites within 10 days when 1200 r were given as whole body irradiation and the animals kept alive during this time by antibiotic and parenteral fluid therapy. 5 animals received fresh, autologous leukocytes obtained by means of a continuous flow centrifuge from the blood. The cell dose was 3.2×10^9 , 7.9×10^9 and in 3 cases 7.5×10^9 mononuclear cells. 5 dogs received in 4 cases 7.5×10^9 and in one case 6.0×10^9 mononuclear cells preserved in DMSO at ultralow temperatures before transfusion. In all instances, the bone marrow showed a marked degree of hemopoietic restauration in the histological bone marrow sections at 10 days. This regeneration commenced (as shown by repeated bone marrow aspirations) at about 5 days, when mononuclear cells were seen in bone marrow and blood that obviously were progenitors of hemopoietic elements. Among these cells a marked fraction showed DNA synthesis as shown by ^3H -thymidine labelling. There were no obvious differences between the effectiveness of fresh and frozen and thawed leukocytes in bone marrow and blood regeneration, when a similar number of mononuclear cells were transfused. In 9 dogs, 1200 r whole body x-irradiation were

given after having collected blood leukocytes during several centrifugation procedures and that were kept frozen until use. This approach yielded a number of mononuclear cells that was between 10.5×10^9 and 39.54×10^9 . When this number of cells were given, a more accelerated bone marrow restoration was seen 10 days after irradiation suggesting a linear cell dose-effect-relationship. At the high cell dose level, the marrow appeared already completely restored. There was an earlier onset of blood leukocyte reappearance when the "high" leukocyte numbers were transfused. - The presence of blood leukocytes in unirradiated dogs endowed with the capability to form granulocytic colonies in soft agar and hence considered to represent granulocytically committed stem cells was confirmed. Such "agar-colony-units" (ACU) reappeared in the blood stream after whole body irradiation and autologous leukocyte transfusion at about 6 days. These findings indicate the feasibility to set up a "blood-stem-cell-transplantation-model" and will allow the quantitative and qualitative characterization of blood stem cells and their regulation.

Ergebnisse des Projektes Nr. 5

Leiter des Projekts und wissenschaftliche Mitarbeiter :

H.D. Flad with Ch. Bruch, W. Calvo, T.M. Fliedner,
E.B. Harriss, E. Herbst, R. Huget, K. von Loringhoven,
H. Meyer, H.P. Schnappauf

Titel des Projekts :

Investigations on the possibility of allogeneic blood stem cell transplantation as a model for the therapy of the acute radiation syndrome

Darstellung der Ergebnisse :

The program deals with the question whether a sufficient number of stem cells can be collected from the peripheral blood of dogs to repopulate the bone marrow of lethally irradiated recipient animals. Since it is known that a graft-versus-host reaction may prevent an allogeneic stem cell engraftment, the degree of histocompatibility between donor and recipient has to be defined in order to work out the cell number necessary for a "take" of the stem cells. Accordingly, the cell number transfused should be large enough to regularly repopulate the marrow of the lethally recipient animal and small enough to obviate or at least mitigate the risk of graft-versus-host reactions ("secondary disease"). Briefly, the experimental design is as follows : leukocytes are collected from the peripheral blood of beagle dogs by means of a IEM cell separator. The cells are frozen at one degree per minute using 10% DMSO as a cryoprotective agent and stored in liquid nitrogen. Dogs undergo the cell separation procedure 3 - 5 times. Recipient littermate dogs are lethally irradiated with 1200 R and transfused with varying amounts ($7 - 30 \times 10^9$) frozen and thawed mononuclear cells. Repopulation of the marrow is followed at intervals by aspiration of bone marrow and histologically after sacrificing the animal at day 10 after irradiation and transfusion.

The results obtained in this program may be summarized as follows:

1. Stem cell transfusions. 2/3 dogs injected with $7 - 8 \times 10^9$ mononuclear cells showed signs of regeneration of the marrow within 7 - 14 days after irradiation. 4/4 dogs receiving $13 - 17 \times 10^9$ mononuclear cells showed marrow regeneration within 6 - 10 days after irradiation. 2 dogs injected with 17×10^9 and 34×10^9 cells respectively developed regeneration

of the marrow within 10 days. Whether there is a correlation between match or mismatch of donor-recipient combinations and cell number inducing marrow regeneration, remains to be established.

2. Histocompatibility testing. Anti DL-A antibodies were raised in dogs by planned immunization with skin grafts. The antisera were tested in the microcytotoxicity test for specificity. Antisera against DL-3, DL-4, DL-5 and two non-DL-A antibodies were obtained. 3 of these sera were found to be operationally monospecific in a workshop held in Rotterdam/The Netherlands in November 1972.
3. Mixed lymphocyte cultures (MLC). The technique of the MLC is at present adapted to dog lymphocytes.
4. Separation of stem cells from immunocompetent cells. The technique of cell separation by means of discontinuous albumin density gradient centrifugation has been adapted to dog peripheral leukocytes. Preliminary experiments suggest that a ten-fold concentration of agar-colony-forming units can be obtained with this technique.

In conclusion, the basic techniques have been developed for a model of allogeneic stem cell transfusions. The next step will be the definition of histocompatibility systems (DL-A, and MLC-loci) in this species and their role for a stem cell therapy. Concomitantly, cell separation procedures will be employed with the aim of concentrating stem cells and eliminating graft-versus-host active, immunocompetent cells. The concept of this model is restricted to its relevance to a stem cell therapy in clinical conditions in man.

Ergebnisse des Projektes Nr. 6

Leiter des Projekts und wissenschaftliche Mitarbeiter :

M. Dietrich (clinical) and H. Heit (experimental) with
T.M. Fliedner, G. Hochapfel, R. Hohage, D. Krieger,
H. Meyer, H. Rasche, E. Vanek

Titel des Projekts :

Investigations on the possibility of bacterial decontamination
in human beings and experimental animals

Darstellung der Ergebnisse (experimental) :

As has been shown before it is possible to render conventional mice bacterialfree by means of poorly absorbable antibiotics (Neomycin, Bacitracin, Pyopen) (report about the research activities 1971). J.M. Jones, R. Wilson et al. reported a prolonged mean survival time in allogeneic and xenogeneic bone marrow chimeras, although a graft-versus-host reaction was found to be present histologically in all chimeras under investigation. In our studies it has been the purpose to investigate, whether observations on mortality from secondary disease similar to those of J.M. Jones et al. can be made if instead of germfree animals as bone marrow recipients "decontaminated" mice are used. All studies were performed with C₅₇ Bl mice as bone marrow donors and CBA mice as lethally irradiated recipients. This combination guarantees a high mortality from secondary disease as C₅₇ Bl and CBA mice differ in all genetic specificities of H-2 locus except at H₂. 50 CBA mice, 8-10 weeks old, were given antibiotics in their drinking water at a concentration of 4 g/l. As a fungistaticum 1 g Pimaricin was added. Sterile food and water was offered ad libidum. From day 3 of antibiotic treatment all bacteriological swabs from feces and bedding material were negative. A secondary contamination of the animals was prevented by maintaining them in a laminar flow banch on sterile containers. Two weeks after the onset of antibiotic treatment all animals (decontaminated and conventional controls) received 800 rad whole body irradiation. Twenty-four hours later 1×10^7 bone marrow cells from female ten weeks old C 57 Bl mice were injected intravenously. All transplanted animals were proved to be bone marrow chimeras by Hb-electrophoresis. Complete chimerism was found to be established from week 7 onwards, first signs were noticed between week 4 and 5.

Fig. 1 shows the different pattern of survival times of decontaminated and conventional chimeras as well as of irradiated conventional and decontaminated mice which did not receive a bone marrow transplantation. All CBA mice irradiated but not transplanted died within the first 3 weeks after irradiation. There was little difference in the survival of decontaminated and conventional mice. No irradiated and transplanted conventional CBA mouse died in the first 3 weeks after transplantation and irradiation. In the 4th week 40%, in the 7th week 80%, and in the 9th week after transplantation, 100% of the animals died of secondary disease. This is the typical pattern of secondary mortality in radiation chimeras of this host-donor combination. Contrary to this findings no decontaminated chimeras died in the 5 week. At the end of the 9th week, when all conventional chimeras were already dead, 80% of the decontaminated survived. At the end of the observation period on day 120, 50% of the decontaminated chimeras were still alive. Clinical signs and symptoms of secondary disease were found to be present in all conventional animals, whereas they were rare or absent in the decontaminated animal group. Results of autopsy showed as a time-dependent the typical manifestations of secondary disease in conventional and decontaminated chimeras. Although the results reported here have to be regarded as preliminary. They suggest the importance of the contribution of the microflora to the course of secondary disease. Further experiments will be designed to show which group of bacteria is responsible for the increased lethality in secondary disease.

Darstellung der Ergebnisse (clinical) :

During 1972, the possibilities and limitations were studied to decontaminate patients with leukemia by means of antibiotics. This study is a randomized trial executed by the Gnotobiotic Project Group with Dr. Dietrich (Ulm) as president and Dr. v.d. WaaJ (Rijswijk) as secretary. 3 groups of patients that are highly susceptible to infection are studied :

- A Patients within an isolation system treated with non-resorbable antibiotics for suppression of the bodies microbial flora.
- B Patients within an isolation system, treated with sterile techniques and meals without antibiotics.
- C Patients treated in a conventional way in open wards.

In Ulm in 1972, 5 patients of group A, 6 of group B and 8 patients in group C were studied intensely. The major question was the fate of the microbial flora and the effect of the remaining bacteria on the organism. Thus, microbial studies were performed on cultures from blood and from external and internal surfaces. Since the randomized trial has not yet been completed, no final conclusions are possible. But from the preliminary impression it appears that isolation has a beneficial effect. Thus, in patients of group A and B, no lung-infections were seen while some patients of group C shown pneumonias with gram-negative bacteria leading to death. In patients of group C, a very interesting observation was made with respect to the appearance of new bacteria not present prior to hospital admission. It showed that the patients in isolation do not acquire as many new bacteria as those in the open ward - the thesis that needed testing and confirmation. These studies are being continued until a total of some 150 patients are being evaluated.

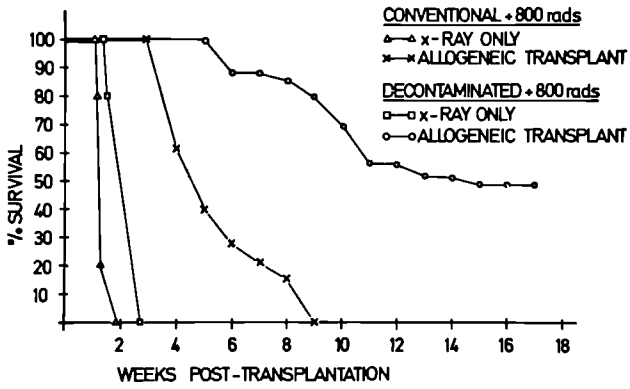


Fig. 1

Survival of decontaminated CBA mice after whole body irradiation and transplantation with allogeneic bone marrow

Ergebnisse des Projekts Nr. 7

Leiter des Projekts und wissenschaftliche Mitarbeiter :

O. Lucarelli (Pesaro) and B. Kubanek (Ulm) with E. Bock,
O. Bock, E.B. Harriss, W. Heit, B. Kubanek, A. Porcellini

Titel des Projekts :

Investigations on the functional relationship between the hemopoietic stem cell pools as a basis for the understanding of hemopoietic regeneration following radiation damage

Darstellung der Ergebnisse :

In 1972, the experimental studies concentrated on the attempt to clarify to some extent the differences observed earlier in the kinetics and regulation of stem cell proliferation and differentiation in newborn and adult rats. This comparison is expected to contribute to the question of the functional relationship between the hematopoietic stem cell pools.

In adults rats, starvation results in a reduction of erythropoietin levels, and a disappearance of erythroid precursors. However, if neonatal rats were fasted for 3 days they still showed some erythroid precursors.

This could indicate a partial independence of neonatal erythroid committed stem cells on erythropoietin or it could be that the cells found after 3 days starvation in the neonatal as in the adult rats.

Erythropoietin is present during red cell production, but predominantly exerts its action at the level of the differentiated erythroid cells by controlling the rate of cell maturation probably through the control of the rate of hemoglobin synthesis. To collect more information on the effect of erythropoietin on red cell production in the normal and starved newborn rat, 2 agents known to affect the rate of cell proliferation and maturation were used, Hydroxyurea (S-phase active), colchicine (mitotically active). It was the purpose of the study to observe if exogenous erythropoietin affected erythroid proliferation or erythroid maturation.

The results (preliminary) indicate that erythropoiesis discontinues as expected within 24 hours when Hydroxyurea is given daily from 7 - 10 days after birth to normal rats and to rats simultaneously fasted for 72 hours. When colchicine was given to 10 day old rats, one group of which were normal controls, another group had been starved from 7th - 10th day of life and a further group starved but treated with 2 units of erythropoietin daily during the time of starvation, several interesting phenomena were observed. It can be deduced from the findings observed that normal erythroid cells are blocked in mitosis by colchicine, following a curve that reaches a peak at 4 hours. The erythroid cells from the starved rats remained unaffected by the colchicine for 10 hours and then starved to accumulate after the 10th hour, peaking at the 16th - 18th hour. When erythropoietin is given to such animals the erythroid population enters mitosis, at a lower rate than normal. The number of mitosis starts to increase at the 2nd or 4th hour reaching the peak at the 12th hour. The data are interpreted to mean that this dose of erythropoietin restores partially the rate of erythroid proliferation in the starved animals and induces then a curve of mitotic accumulation similar to the normal. Further studies will use this methodological approach to study functional relationship between hemopoietic stem cell pools.

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Contractant van de Commissie : Organisatie voor Toegepast
Natuurwetenschappelijk Onderzoek voor Gezondheid (TNO)
Nummer van het contract : 088 - 72 - 1 BIA C

Hoofd van het (de) researchteam(s) :

Prof.D.W.van Bekkum

Algemeen onderwerp van het contract :

Consequences of radiation
exposure, prevention and treatment of pathological effects.

Histocompatibility typing in monkeys has been continued in order to provide suitably typed and matched donors and recipients for bone marrow grafting. Notwithstanding the large numbers of monkeys typed, the yield of phenotypically identical pairs has been low, as are the numbers of available identical siblings. Therefore it has been decided to continue sibling experiments with dogs where tissue typing has now reached the stage that satisfactory solution of genotypically identical siblings is feasible. In that species large numbers of littermates can be obtained relatively fast.

Attempts at morphological identification of stem cells in concentrates from human and monkey bone marrow has continued and light microscopic methods have been under development, aimed at more accurate quantitative examination of stem cells in cell suspensions. For this work and for the evaluation of the various hemopoietic cell preparations employed for grafting, the in vitro culture method for stem cells is being extensively employed. Further work on standardization of media and culture conditions was carried out.

In the mitigation of secondary disease (S.D.), emphasis has shifted from the acute S.D. - which can be largely prevented by the use of purified stem cells or ALS pretreatment of the recipient- to the delayed type of S.D. The main approach has been to explore the influence of infection and the role of the immunological reactivity of the chimeras.

Resultaten van het project No. 1

Hoofd van het team en wetenschappelijke medewerkers :

D.W.van Bekkum and K.A.Dicke/I.Betel

Titel van het project :

Bone Marrow Transplantation

A limited number of monkeys has been grafted with purified stem cells from phenotypically identical non-related donors. The results suggest that the use of such donors causes additional mitigation of the S.D.

In monkey and human bone marrow stem cell concentrates, a morphological entity has been found, which resembles the mouse hemopoietic stem cell in many aspects. This cell has been tentatively described as the hemopoietic stem cell of primates.

The experiments on preservation of mouse, monkey and human stem cells at low temperatures have been completed. Full preservation of stem cell viability is being achieved by employing a stepwise dilution procedure following thawing. This method is now routinely used and is being introduced into clinical transplantations.

The delayed S.D. which develops in allogeneic mouse chimeras and which results in nearly 100% lethality within 3 months, can be largely prevented by modifying the microflora of the recipients. Hardly any mortality occurs in GF animals, as well as in selectively decontaminated mice and in mice with a so-called colony resistant microflora. Moreover, these animals may be conventionalized after 2 months of isolation, without adverse effects. Similar experiments have been initiated with decontaminated monkeys grafted with allogeneic stem cell concentrates.

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Prof. H.J. TAGNON.

Contract n° 088-72-1 BIAC

Dr. P. STRYCKMANS

CELL PROLIFERATION OF LEUKEMIC CELLS.

1. - Factors determining cell proliferation : chemotherapy.

There are several studies presently suggesting that cancer chemotherapy, directly or indirectly, may, in certain conditions, trigger cell proliferation. In human acute leukemia, chemotherapy is apparently able to recruit non-proliferating leukemic blasts, into the cell cycle. A better knowledge of this phenomenon may have practical implications for the treatment of leukemia.

Cytosine arabinoside, vincristine and daunomycin were studied so far. These agents were given to leukemic patients or patients with a generalized tumor, at the usual therapeutic dose.

2. - The process of DNA repair in normal, leukemic preleukemic and lymphomatous individuals.

The recent findings in xeroderma pigmentosum, a human hereditary disease characterized clinically by a high frequency of skin cancer and biochemically by the deficiency of an enzyme necessary in the process of DNA repair, has emphasized the importance of the study of DNA repair processes in relation to cancer.

The capacity for repair of DNA after irradiation with ultraviolet light (50 ergs / mm² delivered in 10 seconds) was studied on cell suspensions, 0.5 mm thick, in tissue culture medium, in the presence of hydroxyurea, after subsequent incubation in the presence of tritiated thymidine.

The uptake of ³H thymidine by the irradiated lymphocytes or leukemic cells was estimated on autoradiographies of blood smears.

Results of Project n° 1.

P. STRYCKMANS, L. DEBUSSCHER, J. MANASTER, G. DELALIEUX

FACTORS DETERMINING LEUKEMIC CELL PROLIFERATION :
CHEMOTHERAPY.

Several parameters of cell proliferation were studied simultaneously and repeatedly before, during and after the administration of chemotherapeutic agents.

- a) The in vitro $^3\text{HTdR}$ labeling index.
- b) The $^3\text{HTdR}$ labeling index of cells labeled in vivo (by one injection of $^3\text{HTdR}$ i. v.) just before the administration of the chemotherapeutic agent.
- c) The mitotic index.
- d) The uptake by cell nuclei of tritiated actinomycin D.
- e) The $^3\text{HTdR}$ uptake per nuclei.
- f) The duration of DNA synthesis by means of a double labeling technique.
- g) The distribution of $^3\text{HTdR}$ flash labeled cells by microdensitometry between $2n$ and $4n$ nuclear DNA content.

Cytosine arabinoside (100 mg/m^2 BSA) once or repeatedly.

So far, 6 patients have been studied with cytosine arabinoside : 4 had acute myeloblastic leukemia (AML) and 2 were in a leukemic transformation of a lymphosarcoma (LLS).

One patient only, with AML, showed a significant increase of (a), (c) and (d). The other parameters are still under study. The increase was seen in the bone marrow cells but not in the blood.

In the other patients (a) decreased and so did (d).

In one of these patients (LLS), the above mentioned parameters could be studied simultaneously on blastic cells in 4 different locations (marrow, blood, lymph node and pleural effusion). Significant depression of (a) was observed in all the samples.

Vincristine (1 or 2 mg/m² BSA).

The effect of VCR was studied on :

- normal myeloid and erythroid cells (from the bone marrow of a generalized cancer patient ;
(a) and (c) increased within 24 hours.
- Leukemic lymphoblasts (from the blood of a patient with acute lymphoblastic leukemia) ;
no increase of (a) and (d) was observed.

Daunomycine (60-80 mg/m² BSA).

Its effect was studied on the blastic cells from the bone marrow of 4 patients with AML.

In one patient, a significant increase of (a) and (c) was observed.

These preliminary results suggest that in some cases the flux of normal myeloid cells and leukemic cells entering DNA synthesis may be increased as a consequence of the administration of chemotherapeutic agents. Complementary studies are going on to rule out the possibility that the observations made do not simply reflect the blockage of cell in DNA synthesis.

Results of project n° 2.

P. STRYCKMANS, G. DELALIEUX and J. MANASTER.

THE PROCESS OF DNA REPAIR IN NORMAL, LEUKEMIC AND LYMPHOMATOUS INDIVIDUALS.

The uptake of ^3H thymidine by the irradiated lymphocytes or leukemic cells was estimated on autoradiographies of blood smears.

The lymphocytes of 18 normal individuals showed over their nucleus between 200 and 450 silver grains/100 lymphocytes.

The lymphocytes of 4 normal infants showed labelings into the limits for normal adults.

The lymphocytes of patients with high risk for acute leukemia (2 with mongolism - 1 with Fanconi anemia) showed also normal values.

The lymphocytes of 4 patients in complete remission of acute lymphoblastic leukemia showed normal values.

The lymphocytes of 1 patient with Hodgkin's disease had normal values.

The lymphoblasts of 3 patients with acute lymphoblastic leukemia showed significantly decreased values (all were below 200).

There is no indication so far for a decreased capacity to repair DNA in the lymphocytes of leukemic patients. Leukemic lymphoblasts, generally considered as belonging to the lymphoid cell line, seem to show a decreased capacity to repair. This study is still in progress.

1. P. STRYCKMANS, G. DELALIEUX, J. MANASTER and M. SOCQUET.
The potentiality of out-of-cycle acute leukemic cells to synthesize DNA.
Blood 36 : 697, 1970.

2. J. MANASTER, J. FRUHLING, P. STRYCKMANS.
Kinetics of lymphocytes in chronic lymphocytic leukemia.
I. Equilibrium between blood and a readily accessible pool.
Blood (in press) (March 1973).

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung,
Institut für Hämatologie

Nr. des Vertrags: 089-72-1 BIAD

Leiter der Forschungsgruppe:

Priv.-Doz. Dr. Stefan Thierfelder

Allgemeines Thema des Vertrags:

Strahlenbiologische Hämatologie und Immunologie. (Proj. 1-5).

(Proj. 7-12 über Nuklearmedizinische Hämatologie sind unter Kapitel V "Forschungstätigkeit Anwendungen Medizin" aufgeführt. Am Schluß dieses Teils des Berichtes befindet sich eine Aufstellung der Publikationen.)

The immunological department of the institute concentrated on questions concerning the treatment of the consequences of radiation exposure. It isolated a fraction active against T-cells from heterologous antibrain serum, which suppressed acute secondary disease (project nr. 5) and produced T-cell deprived mice simulating the nude strain (proj.nr.3). Furthermore it documented the low degree of chimaerism in mice conditioned with cyclophosphamide (proj.nr.2) and developed a non-lethal conditioning treatment of partial body irradiation and ALS (proj.nr.4).

In radiation hematology the long term studies on the morphological consequences of a treatment with radiophosphorus were continued (proj.nr.1). The basic studies of a new in vitro method for determining DNA synthesis rates of individual, morphologically classifiable human cells have been completed and applied to various forms of human anemia (proj.nr.10).

Ergebnisse des Projekts Nr. ..1.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Prof.Dr.R.Burkhardt und Dr.E.Beil

Titel des Projekts :

Bone marrow histology in patients treated with radiation,
isotopes and radiomimetic agents.

1. A previous study on the bone marrow changes in polycythemia vera has been continued. The first results were published in 1969 (see 1887/III/71-D No.2). 12 patients of this group were investigated once more last year. 32 patients entered in the program. The number of the patients treated repeatedly with radiophosphorus during a period of more than ten years and controlled by biopsy with special histological methods more than three times is now 9. One of the whole group developed lymphocytic leucemia, 3 patients developed granulocytic leucemia and 13 patients myelofibrosis. The study is to be continued. Another statistical evaluation of the results will be accomplished within the next years.

2. Qualitative and quantitative studies of the role of the megakaryocytes of the bone marrow in polycythemia vera during treatment with radiophosphorus have been concluded with the following results: Excessive proliferation of the megakaryocytes is to be found. Compared with an increase due to stimulated regeneration, the pycnotic and necrotic changes of these cells are much more prominent in polycythemia. In the cases without treatment the absolute numbers of megakaryocytes increase in the course of time. This is true especially for active and inactive forms in close position to the marrow sinusoids. Treatment with ^{32}P blocks these changes for as long as 3 to 28 years, when applied in regular intervals. Chemotherapy with Busulfan or TEM has a similar effect. However, this also causes a considerable rise in the number of pycnotic and necrotic cells. There is no significant increase of fibrotic marrow changes in the group which has been treated with ^{32}P in contrast to the group treated with chemotherapy. These results may contribute to understanding the antithrombotic effect of the therapy with ^{32}P and the pathogenesis of marrow fibrosis in polycythemia.

3. In leucemia of different types investigations of the histological changes of the bone marrow during therapeutical remission, relapse and final collapse have been started. Until now 20 patients entered in this study. In 8 of the cases it was possible to control the changes by repeated biopsies until death. The study is to be continued.

4. The instant and the late effects of a well defined dosage of nuclear energy on a distinct osseous region are to be investigated quantitatively in a human population with adequate bioptic and histologic mehtods. In 11 women suffering from genital malignoma, treated with radiation from a Ra²²⁶ source, a histobiopsy of the iliac crest was taken before the procedure after a dosage of 1000 R and after 3000 R. The effective dosage in the area of the biopsy has been calculated. In another group of 10 women the late effects of the radiation will be sutdied at an interval of 6 months from the end of the therapy in the same way. The main aim of this sutdy is to collect information on the following subjects : Changes of the bone marrow capillaries, the reticular tissue and the type of regeneration of the marrow cells after the therapeutic marrow depletion. The study will be finished within three months.

Ergebnisse des Projekts Nr. .2.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
S.Thierfelder, v.Arnim, P.Baumann

Titel des Projekts :
Degree of chimaerism following different types of conditioning treatment

Various criteria have been used to prove engraftment in conditioned recipients of bone marrow. Cytogenetic differences between host and donor are particularly useful because the number of dividing donor and host cells can be quantitated more precisely than serologic differences. The present investigations concern the degree of chimaerism obtained after 200 and 300 mg/kg of Cyclophosphamide or 40 mg of Myleran applied to syngeneic (CBA; CBA-T66) or semiallogeneic (C57Bl/6 x CBA) F₁ mice 6 or 24 hrs before transplantation. Fig. 1 shows that less than 10 % of the donor metaphases were found 11, 21 and 56 days after syngeneic transplantation, while the more actively dividing donor cells in the semiallogeneic donor-recipient combination with homologous disease remain around 10 %.

The low degree of chimaerism following 300 mg/kg of Cyclophosphamide in syngeneic and in combinations with florid gvH tend to reconcile Santos success to induce homologous disease after cyclophosphamide with Van Bekkums difficulties to induce chimaerism in primates.

Cyclophosphamide is known to have a lower stem cell toxicity compared to irradiation. It is therefore less appropriate for the induction of stable chimaerism in non-aplastic bone marrow recipients. The effect of ALS and irradiation as well as their combination with Cyclophosphamide and Busulfan is under investigation.

The high degree of almost 100 % syngeneic chimaerism after Busulfan should be expected from results obtained by Floersheim and others with other techniques.

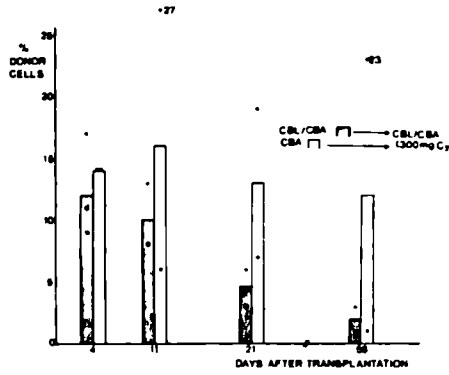


Fig. 1: Donor metaphases in syngeneic and semiallogeneic (parent-to-F₁ hybrids with homologous disease) recipients conditioned with 300 mg/kg Cyclophosphamide and transfused with 5 x 10⁷ spleen cells.

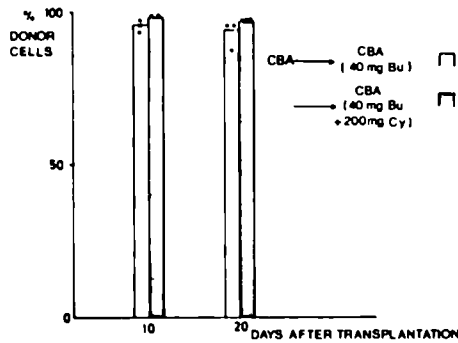


Fig. 2: Donor metaphases in syngeneic recipients conditioned with 40 mg Busulfan alone or with 40 mg Busulfan and 200 mg Cyclophosphamide.

Ergebnisse des Projekts Nr. 3.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Priv.Doiz.Dr.S.Thierfelder, Dr.Rodt, Dr.Thiel

Titel des Projekts : Irradiation and other immunosuppressive
procedures in mice in order to obtain a state simulating the
complete lack of cellular immunity of thymusless mice

The nude mouse is the rare in-vivo model for the study of tolerance of histoincompatible skin grafts. In addition it provides us with hemopoietic stem cells (almost) completely lacking T-cells. It should therefore be an ideal marrow donor for incompatible recipients provided acute as well as chronic secondary disease is initiated by T-cells of the donor. This assumption was tested and found to be valid: spleen cells of nude mice derived from a nude stock backcrossed to Balb/c H-2d was transferred into lethally irradiated CBA-J H-2d. While spleen cells of heterozygous Balb/nu parental donors caused fatal acute secondary disease, the majority of recipients of homozygous nu/nu spleen cells survived.

A handicap in this system is the lack of information on the exact degree of histoincompatibility when one uses nu/nu mice, because the histocompatibility antigens of nu/nu mice are less well defined, in our mice for example only 75 % Balb/c. We therefore tried to obtain T-cell deprived mice from a well defined strain.

CBA mice irradiated with 900R and reconstituted with bone marrow from CBA donors rejected skin grafts of C57Bl/6 donors H-2b within 25 days. The 50 % survival of these H-2 incompatible skin grafts was prolonged to 36 days when the recipient CBA mice were thymectomized before marrow transplantation. It was however clear that murine bone marrow reconstituted lethally irradiated and thymectomized recipients with sufficient T-cells to reject strongly H₂ incompatible skin grafts. We therefore incubated bone marrow with heterologous anti-T-cell serum (rabbit-anti-mouse-brain serum, thoroughly absorbed with murine liver and plasmocytoma) and injected it into syngeneic thymectomized recipients. These animals tolerated C57Bl/6 skin grafts

beyond the observation period of 3 months. They lost weight gradually while the non-thymectomized controls (recipients of syngeneic marrow incubated with anti-T-cell serum) gained weight and 50 % of them rejected C57Bl/6 skin grafts within 36 days.

Thymectomized recipients of syngeneic bone marrow treated with heterologous anti-T cell serum therefore appear to lack cellular immunity to a degree comparable to the nude mouse and will now be used to study the T-cell dependence of chronic secondary disease.

Ergebnisse des Projekts Nr. 4.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
S.Thierfelder, H.Kolb, W.Mempel

Titel des Projekts :
Partial body irradiation and immunosuppression with ALS

The preparation of a bone marrow recipient to accept the foreign hemopoietic graft, i.e. conditioning treatment, usually consists of either irradiation or immunosuppression with cydophosphamide or anti-lymphocytic serum (ALS). Unfortunately, irradiation or cydophosphamide have to be employed at doses which can cause life-threatening complications. Only ALS is comparatively harmless provided it is free of antibodies cross-reacting with antigens on kidneys or platelets. ALS on the other hand lacks stem cell toxicity in vivo necessary to make syngeneic or allogeneic recipients with intact haemopoiesis accept foreign bone marrow. Since the induction of chimaerism in order to produce specific tolerance of organ transplants would usually deal with individuals having a normal haemopoiesis we developed a murine model for conditioning treatment consisting of a 6 days' course of 0.25 ml/day of ALS and a partial body irradiation of 900 R applied to the lower half of the body including the spleen thereafter. This combined treatment is not lethal even without the transplantation of marrow. It induces a stable chimaerism of syngeneic and allogeneic H₂-compatible (C₃H-to-CBA, CBA-to-C₃H) cells. 70-90 % donor metaphases were found in the irradiated parts of the spleen and femur while only about 15 % of the metaphases of the recipients' shielded parts were of donor type. There was no allogeneic chimaerism when ALS was omitted from the partial body treatment.

Allogeneic H₂-incompatible haemopoietic grafts persisted during the observation period of 50-100 days provided the donor differed from the recipient by 1 haplotype only.

Thus ALS combined with partial body irradiation represents a non-lethal conditioning treatment which induced syngeneic, allogeneic H₂ compatible, and semiallogeneic H₂ incompatible chimaerism in the mouse.

Ergebnisse des Projekts Nr. .5.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
H.Rodt, S.Thierfelder, M.Eulitz

Titel des Projekts :

Suppression of acute secondary disease with T-cell specific antisera

In the course of our experiments on enhancement and allogeneic inhibition in T-cell deprived mice (Proj.No.3), the original topic of this investigation was put aside in favour of a more detailed study on the consequences of T-cell deprivation with heterologous T-cell specific antisera. It was found that such antisera completely suppressed acute secondary disease (ac.sec.dis.)

Ac.sec.dis. - measured in the murine parent-to-F₁ model - has been reported to be effectively suppressed by either donor treatment with ALS or the gradient centrifugation and fractionation of spleen marrow of the donor. The first approach risks untowards effects of the injection of heterologous sera contaminated with unwanted antibodies while the latter loses stem cells, which can become critical in the primate. Our approach consists in a 20 minutes' incubation of 5 or 50 x 10⁶ parental spleen cells with rabbit-anti-mouse \emptyset -serum and subsequent injection into lethally irradiated H-2 incompatible F₁ hybrids. 90 % of the recipients survived the observation period of 100 days as complete chimaeras without symptoms of wasting. All the controls treated with rabbit-anti-mouse IgG were dead 20 days after transplantation.

The anti-T-cell serum was produced by injecting rabbits with mouse brain, subsequent absorption with liver and plasmacytoma and re-concentration of the IgG fraction. The purified fraction reacted against cells of spleen and thymus at a cytotoxic and complement-fixing titer of about 1 : 16. It was negative with spleen cells of athymic nude mice. In contrast to Golub who found that absorbed anti-brain sera crossreacted with an antigen on colony-forming stem cells, which would of course disqualify them as a means to suppress secondary disease, our antisera appeared to spare stem cells in the spleen colony-forming test.

It should be pointed out that anti-brain serum, which so easily eliminates acute secondary disease, only delayed chronic secondary disease. This tends to support van Bekkum's hypothesis that chronic secondary disease differs qualitatively and not only quantitatively from ac.sec.dis. and is the result of undefined precursor cells. Our model seems useful to test this hypothesis in more detail. It may also help in secondary disease of recipients of weaker incompatibilities (L-A identical individuals)

Associazione CNEN-Euratom
Contratto No. 108-72-1 B101

G. DORIA

IMMUNOGENETICS

The immune system protects the individual from invasion and pathogenic action of microorganisms. Efficiency of the immunologic surveillance relies on integrity of the populations of immunologically competent cells that participate in the processes of antigen recognition and antibody production. Several immune responses require the cooperation between 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 affected by antibody avidity, a function of affinity, which determines the stability of the antigen-antibody complexes.

The increase of antibody avidity in the serum with time after immunization and with lowering the antigen dose has been attributed to selective pressure exerted by antigen concentration at the level of antigen-sensitive cells. According to the maturation theory, the precursors of cells producing antibodies with low affinity need higher concentration of antigen to be triggered to antibody formation than the precursors of 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. This view is supported by the finding that the precu-

sors of antibody-forming cells possess membrane-bound receptors with a wide range of affinities and that the receptors of each precursor cell have the same affinity as the antibody secreted upon the combination of antigen with the receptors.

The effect of the helper function of T cells may be selective activation of B cells with low affinity receptors by a mechanism of antigen concentration. Alternatively, T cells may release mitotic factors that amplify the B cell population with no selection for affinity. Finally, T cells may preferentially stimulate B cells with high affinity receptors by some entangled mechanism.

The protective value of antibody avidity becomes mostly apparent whenever the immune system is altered, as a consequence of exposure to radiations or of abnormal recovery by transplantation of allogenic hemopoietic cells. Knowledge of how antibody avidity is determined at the cellular level is relevant to the understanding of immune functions not only in normal individuals but also in radiation chimeras for which donor cell manipulations can be attempted to improve survival of the irradiated host.

A technique has been devised whereby antibody avidity can be assessed at the level of single immunocytes under several experimental conditions. Mice were immunized with TNP-HRBC (trinitrophenyl-horse red blood cells), a hapten-carrier conjugate. The immune response of spleen cells to TNP was assayed by the Jerne technique with TNP coupled to SRBC, a non-crossreactive carrier, and expressed as number of direct PFC. If spleen cells were plated with TNP-SRBC and TNP-BSA (trinitrophenyl-bovine serum albumin), PFC were inhibited. The amounts of inhibitor added were referred to mg of TNP-lysyl residues estimated from spectrophotometric measurements of the TNP-BSA solution. The percent inhibition of PFC increased as a sigmoid function of the log amounts of inhibitor added. From probit analysis of the data and antilog transformations the ED_{50} (median effective dose of inhibitor that suppresses 50% of the PFC) and its 95% confidence limits were calculated. The reciprocal of ED_{50} (mg^{-1}) was taken as an estimate of avidity: the higher the antibody avidity, the lower the ED_{50} and the higher

its reciprocal.

In each experiment mice were injected i.v. with 4×10^8 , 4×10^6 , or 4×10^4 TNP-HRBC and groups of animals were sacrificed daily for one week after each antigen dose. The spleens of each group were individually tested for the number of PFC anti-TNP and, after they were pooled, for the antibody avidity at the level of single PFC. The number of PFC per spleen rapidly increased from the background level to a peak value on day 4 after immunization and declined thereafter. These changes in PFC number were paralleled by a rise and fall, up to 60 fold, of antibody avidity. Avidity displayed a similar pattern of variation, regardless of changes in antigen dose by a factor of 10^4 . Furthermore, both the number of PFC per spleen and antibody avidity decreased with lowering the antigen dose.

Similar results were obtained in vitro, where mouse spleen cells could be stimulated with TNP-HRBC to produce PFC anti-TNP inhibitable by TNP-BSA.

The increase of avidity with antigen dose and its rise and fall with time seem at variance with the theory of maturation in affinity of the antibody response. The following tentative explanation is proposed. Cell selection is influenced by antigen dose and also by the presence of secreted antibodies. The antibody competes with the cells for antigen and thus provides a selective drive in favor of high-affinity cells which can capture free antigen in low concentration and be stimulated to divide and synthesize antibodies. The rate of increase of the PFC number was found positively related to the antigen dose, suggesting that higher doses were a more intense stimulus for antibody production. Thus, the higher the antigen dose the faster the rate of increase of antibodies exerting a selective drive in favor of the higher-affinity cells. This would explain the increase of avidity observed when the antigen dose was varied by a factor of 10^4 . During the first 4 days after each antigen dose avidity was

found to increase because the antibody concentration was increasing so that less antigen was remaining available for cell stimulation. From day 4 on, the concentration of antigen dropped to a very low level at which statistical factors might play an important role in determining the outcome. That is, whether a particular cell is stimulated depends not only on its affinity for antigen but also on its chance of coming in contact with one or more antigenic determinants. Since at the onset of the immune response antibody affinities in the serum seem to be normally distributed, one can assume that also the affinity of antigen-sensitive cells follows the same distribution. Thus, if there are far more cells of average affinity present than of very high affinity, when the antigen concentration is extremely low one antigenic determinant is more likely, on a purely chance basis, to hit a cell of average affinity than of very high affinity. Such statistical considerations could explain the fall of avidity observed after day 4. This provisional interpretation can be submitted to experimental trial.

PUBLICATIONS APPEARED IN 1972

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Contractant de la Commission :
UNIVERSITE LIBRE DE BRUXELLES

N° du contrat : 093-72-1 BIOB

Chef du groupe de recherche :

Jacques E. DUMONT

Thème général du contrat : Définition de la méthodologie pour l'étude des effets des radiations sur les tissus humains (sang, etc.) et application de cette méthodologie.

Le but général du projet est l'étude de mécanismes physiologiques et biochimiques dont l'altération détermine des effets à court et à long termes des radiations et de définir les méthodologies permettant d'étudier ces mécanismes. Les domaines investigués sont la régulation de l'érythropoïétine, le leucocyte et sa capacité de phagocytose et l'établissement d'un modèle de l'irradiation des cellules folliculaires thyroïdiennes après ingestion de divers radioisotopes de l'iode.

A) Erythropoïétine

L'irradiation affecte le métabolisme de l'érythropoïétine.

Pour étudier cet effet, le métabolisme de l'érythropoïétine a été exploré. Il a été démontré que :

- le rein a un rôle majeur dans le catabolisme de l'homme ;
- l'explication de la courbe de disparition à deux composantes de l'érythropoïétine plasmatique n'implique pas l'hypothèse de deux érythropoïétines différentes ;
- la dépression de la réponse érythropoïétique chez l'animal à jeun est due à une absence de réponse à l'érythropoïétine.

B) Phagocytose et destruction des bactéries par le leucocyte

L'irradiation entraîne une leukopénie et une réduction qualitative de l'activité bactéricide et de phagocytose du leucocyte. Nos résultats suggèrent que la 3',5'-AMP cyclique n'est pas le médiateur intracellulaire des conséquences métaboliques de la phagocytose. Les leucocytes d'un patient atteint de granulomatosse chronique ont été étudiés comme modèles de leucocytes de patients irradiés. Il a été démontré que certains agents oxydants (tel le bleu de méthylène) corrigent l'insuffisance de la capacité bactéricide du leucocyte.

C) Modèle du métabolisme de l'iode dans la thyroïde

Ce programme a été poursuivi en dehors du cadre du contrat en 1972.

Résultats du projet n° .I..

Chef du projet et collaborateurs scientifiques:

J.P. NAETS M. WITTEK

Titre du projet :

Effet de l'irradiation sur le métabolisme de l'érythropoïétine.

Deux effets de l'irradiation sur l'hématopoïèse sont connus : la dépression générale de l'hématopoïèse et le ralentissement du métabolisme de l'érythropoïétine (Proc. Soc. Exper. Biol. Med., 100, 40, 1959). Notre étude porte sur ce second aspect. Notre hypothèse est que des rayons X agissent sur le rein qui serait le principal site de catabolisme de l'hormone.

A) Métabolisme de l'érythropoïétine

Nous avons poursuivi les études sur le rôle du rein dans le catabolisme de l'érythropoïétine. De nombreuses expériences réalisées en 1972 confirment que le rein est indispensable au catabolisme de l'hormone. Un T/2 allongé (10h30) est en effet trouvé chez l'animal anéphrique, alors qu'un T/2 semblable à celui des témoins normaux (2h) est observé chez l'animal à uretères ligaturés. Ces résultats ont été obtenus aussi bien avec le plasma de rat hypoxique (ayant un taux élevé d'érythropoïétine), qu'avec de l'érythropoïétine d'origine ovine (Armour). La courbe de décroissance observée chez les néphrectomisés suit une double exponentielle que l'on peut décomposer en une composante rapide (T/2 = 30'), et une composante lente (T/2 = 10h30). Nous avons au départ émis l'hypothèse que cet aspect particulier de la courbe correspondait à la présence de deux formes d'érythropoïétine : l'une rapidement métabolisée, même en l'absence de rein, l'autre, plus lentement métabolisée chez l'animal anéphrique. Cette hypothèse a dû être abandonnée à la suite des résultats suivants : au lieu d'injecter aux rats néphrectomisés du plasma de rat prélevé immédiatement

après l'arrêt du stimulus hypoxique, nous avons utilisé du plasma prélevé 2h après la stimulation hypoxique, théoriquement débarrassé de la "composante rapide", si notre hypothèse était exacte. Nous aurions en ce cas dû observer un T/2 correspondant uniquement à la composante lente. En fait, nous avons retrouvé une courbe à deux exponentielles, voisine de celle obtenue avec le plasma prélevé à la sortie du caisson. Nous supposons donc que la ^{1ère} partie de la courbe correspond à la répartition de l'érythropoïétine dans un espace plus grand que le compartiment plasmatique.

B) Effets du jeûne sur la réponse à l'érythropoïétine

Au cours du jeûne, le métabolisme chez le rat tombe de 30% et l'on considère qu'en réponse à ces besoins réduits en oxygène, la production de l'érythropoïétine diminue. Dans la littérature, on admet que la réponse à l'érythropoïétine exogène est normale chez l'animal à jeûn, or il s'agit en fait de travaux concernant des rats carencés en protéines, non à jeûn. Nous avons étudié la sensibilité à l'érythropoïétine du rat à jeûn, normal et polyglobulique. Nos résultats montrent une nette réduction de la réponse à l'érythropoïétine chez les animaux à jeûn. Cette réduction se marque aussi bien sur l'incorporation du Fe⁵⁹ dans les hématies, que sur la réticulocytose. C'est ainsi que l'injection de 2 unités d'Eine IV élève l'incorporation de Fe⁵⁹ à 24,98% chez le rat polycythémique nourri normalement, mais n'est que de 4,42% chez le rat polycythémique à jeûn.

En conclusion, il semble bien que la dépression de la réponse érythropoïétique observée au cours du jeûne soit essentiellement liée à l'absence de réponse à l'érythropoïétine plutôt qu'à une production diminuée de l'hormone, contrairement à ce qui était généralement admis.

Publication

NAETS, J.P. et WITTEK, M. : The kidney and utilization of erythropoietin.
Separatum Experientia, 27,1468,1971

Résultats du projet n° .II.

Chef du projet et collaborateurs scientifiques:
E. SCHELL-FREDERICK, J. VAN SANDE, J.E. DUMONT

Titre du projet :
Leucocyte et Phagocytose.

L'efficacité physiologique des leucocytes polymorphonucléaires dans la défense de l'organisme est fonction de la concentration et de l'activité de ces cellules dans le sang. L'irradiation entraîne une leukopénie bien connue, mais aussi une réduction importante qualitative de l'activité phagocytaire et bactéricide du leucocyte (Nature, 210, 158, 1966 ; J. Reticuloendoth. Soc., 5, 538, 1968 et 7, 743, 1970). Dans ce travail, nous étudions le mécanisme de ces deux activités pour appliquer cette connaissance à l'irradié. Deux aspects ont été envisagés : une affection génétique, la granulomatose chronique (CGD), qui pourrait être considérée comme un modèle de la lésion d'irradiation, et le rôle de la 3',5'-AMP cyclique.

A) Le modèle de la granulomatose chronique (CGD)

Cette affection génétique portant sur la fonction du leucocyte est caractérisée cliniquement par des affections pyogéniques récurrentes conduisant à une mort précoce. Les leucocytes phagocytent normalement les bactéries mais ne les détruisent pas; ils ne présentent pas les concomitantes métaboliques de la phagocytose (stimulation de la voie des pentoses phosphates, de la formation d' H_2O_2 et de l'iodation). La mortalité élevée dans les infections postirradiations indique dans ce cas aussi une activité bactéricide réduite.

Méthodes : Des leucocytes de volontaires normaux, de patients présentant des infections aiguës, d'un patient présentant une granulomatose chronique (liée au chromosome X) et de porteurs hétérozygotes ont été préparés et étudiés comme décrit précé-

demment (Euratom Research Report, 1971, II, 636). L'irradiation a été mesurée d'après Pincus et Klebanoff (New Engl. J. Med., 284, 744, 1971), le pouvoir bactéricide d'après Mandell et Hook (Am. J. Med., 47, 473, 1969).

Résultats : Bien que le défaut enzymatique de la cGD n'ait pas été complètement défini, il apparaît clairement que l'altération métabolique fondamentale de ces leucocytes est leur incapacité à former l' H_2O_2 nécessaire à l'inactivation bactérienne. Si H_2O_2 est introduit dans le leucocyte affecté, la stimulation par la phagocytose de la voie des pentoses phosphates, de l'oxydation du formiate et de l'iodation réapparaissent. Nous avons essayé de corriger le défaut par l'administration d'agents oxydants. La mesure de l'iodation au cours de la phagocytose est un test biochimique du pouvoir bactéricide de l' H_2O_2 leucocytaire. Dans les leucocyte CGD, des particules de latex produisent une stimulation faible (X2) de l'iodation (X10 à 20 chez les normaux). Le bleu de méthylène (5 à 100 μ M) dans ces conditions multiplie l'effet du latex (X5 à 10). La vit K_3 est moins efficace. Des résultats préliminaires suggèrent que ces composés ont un effet identique sur le pouvoir bactéricide per se des leucocytes. Des essais in vivo sont envisagés. Ces résultats seront rapportés au 7ème Congrès de la Société Européenne d'Investigation Clinique (Pays-Bas, avril 1973).

B) Role de la 3',5'-AMP cyclique dans la phagocytose

Les expériences rapportées en 1971 ont été confirmées et étendues. La stimulation fugace de la formation de 3',5'-AMP cyclique dans le leucocyte n'explique qu'une des concomitantes métaboliques de cette phagocytose, la glycogénolyse accrue mais non les autres (activation de la voie des pentoses phosphates, de l'oxydation du formate, de la respiration, de l'iodation, etc.). Le rôle d'autres médiateurs intracellulaires (3',5'-GMP cyclique, Ca^{++} , etc.) devra être envisagé.

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 : 095-72-1BIOB

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.

LES EFFETS A COURT TERME

Dans ce cadre nous avons groupé deux recherches :

la première : sur les indicateurs biochimiques des lésions des radiations et notamment sur le métabolisme de la déoxycytidine, du NAD, de l'acide sialic et de la sérotonine ;

la deuxième : sur les mécanismes de réparation du DNA mitochondrial.

LES EFFETS A LONG TERME

Nous décrivons sous cette rubrique nos résultats :

- 1) Sur l'influence des radioprotecteurs sur le raccourcissement de la vie, sur l'incidence des leucémies et des cancers et les effets non néoplasiques des radiations ;
- 2) Sur les effets des radiations sur la biochimie du cerveau (contenu en DNA, protéines, acide sialic, β -glucuronidase, acide phosphatase, cathepsine, sérotonine, histamine, etc...).

LES EFFETS GENETIQUES

Nous résumons sous cette rubrique les résultats de 3 projets de recherches.

- 1) Les remaniements chromosomiques produits par les rayons-X dans les cellules reproductrices des souris mâles.
- 2) Les remaniements chromosomiques produits par une irradiation des cellules reproductrices femelles.
- 3) Les anomalies chromosomiques produites par les irradiations au moyen des techniques de fluorescence et de "banding pattern".

Résultats du projet n° 1

Chef du projet et collaborateurs scientifiques :

G. GERBER, J. DEROO et J.P. DECOCK

Titre du projet : BIOCHEMICAL INDICATORS OF RADIATION DAMAGE

1. The investigations dealing with the metabolism of deoxycytidine after irradiation were completed. Its metabolism was followed in the intact mouse and rat as well as in isolated perfused rat liver, rat intestine and mouse liver. A technique to perfuse mouse liver was developed to this end. Although deoxycytidine is rapidly degraded in mouse as in man, this loss balances about that incurred by the rat due to excretion in urine. Consequently, metabolism of DNA differs not much between these species.
2. The experiments dealing with metabolism of NAD were also completed in this year by following metabolism of nicotinamide in intact animals and isolated perfused liver. The changes after irradiation are explained on the basis of increased NAD catabolism not compensated for by resynthesis.
3. Content and excretion of free and bound sialic acid was studied after whole body irradiation. Excretion of sialic acid displays a dose dependent increase on the first day. Other experiments dealt with metabolism of pseudouridine after irradiation for which a new technique of determination in urine was developed. Haptoglobin levels in blood were also followed after irradiation. This serum protein increases as early as 5 hours after lethal exposure and continues to increase during the post-irradiation period. Other studies on biochemical indicators of radiation damage were started on urine of irradiated monkeys in cooperation with the Institute of Rijswijk.
4. Serotonin is thought to play an important role in the radiation syndrome. Using isolated perfused organs (liver and intestine) we followed synthesis of serotonin from 5-OH-tryptophan and its subsequent catabolism in intestine during the early phase of the G.I. syndrome (after 2 kR) and in liver prior death from the bone marrow syndrome (8 days after 1000 R) at normal and elevated substrate levels. Catabolism of serotonin was found increased, and its synthesis from 5-OH-tryptophan also exceeded that of non irradiated controls under both experimental conditions.

Excretion of the catabolite 5 OH indolacetate was followed in male and female rats and mice. In agreement with published data in rats, this excretion was found increased early after exposure although less so than had been described by others.

5. Studies on neonatal irradiation and ageing.

This investigation was undertaken in order to learn whether :

- a. The marked retardation in growth after neonatal irradiation in the mid-lethal range (400 R) is related to an altered uptake of substrate (e.g. an amino isobutyrate (AIB)) by organs and/or to changes in brain biochemistry (content on DNA, proteins, sialic acid, β -glucuronidase, acid phosphatase, cathepsine, serotonin, histamin, gamma aminobutyrate, catecholamines).
- b. To which extent these parameters depend on age, strain and sex of the animals. The most important observations obtained so far are : uptake of AIB by liver increases, that by muscle and brain decreases with age. Neonatal irradiation depresses uptake of AIB by brain. Serotonin, cathepsin and acid phosphatase increase, β -glucuronidase decreases with age. The activity of β -glucuronidase depends markedly also on the strain of animals. Neonatal irradiation depresses among others permanently the DNA content of the brain i.e. the number of cells. On the other hand, local irradiation (2 kR) of adult brain increased DNA, serotonine and sialic acid but also affect^{ed} uptake of AIB by muscle.

- G.B. GERBER, A. DECLEVE, J.R. MAISIN, A. LEONARD and Gh. MATTELIN
Fate of DNA labeled bone marrow cells from different mouse strains injected into syngeneic irradiated recipients.
Experientia : 28, 149-151, 1972.
- G.B. GERBER and J. DEROO
Metabolism of hydroxytryptophan in isolated perfused rat liver.
Z. Klin. Chemie Klin. Biochem. 10, 177, 1972.
- G.B. GERBER, B. ZICHA, J. DEROO, J.P. DE COCK and E. GEYER
Metabolism of deoxycytidin in isolated perfused organs.
Biophysik 8, 333-342, 1972.
- G.B. GERBER, J. DEROO, J.P. DE COCK
Metabolism of deoxycytidine in different organs of mice and rats.
Arch. Internat. Physiol. Biochem. 80, 353-365, 1972.
- G.B. GERBER, J. DEROO,
Metabolism of NAD after whole body irradiation IV Incorporation of nicotinanide by isolated perfused liver and by intact rats.
Int. J. Rad. Biol. 22, 351-360, 1972.
- A. DECLEVE, G.B. GERBER, A. LEONARD, M. LAMBIET-COLLIER, A. SASSEN, J.R. MAISIN
Regeneration pattern of thymus, spleen and bone marrow in X-irradiated AKR mice with or without syngeneic bone marrow transplantation.
Rad. Research : 51, 318-331, 1972.
- G.B. GERBER
Biochemical indicators of radiation damage. A perspective and a review.
J. Belge de Radiol. (in press).
- G.B. GERBER
Biochemical and molecular-biological endpoints in late effect studies.
Essler 2nd Symposium, Rome, Sept. 29, 1972. (in press).

Résultats du projet n° 2

Chef du projet et collaborateurs scientifiques :

L. BAUGNET-MAHIEU, A. SHIMA^x, R. GOUTIER et C. BAES

Titre du projet : MECANISMES DE REPARATION DU DNA MITOCHONDRIAL

Nous avons décrit, dans le rapport précédent, nos premiers résultats sur l'étude de la réparation du DNA mitochondrial dans des suspensions de mitochondries irradiées in vitro. Deux phénomènes ont été mis en évidence; ils accompagnent les processus de réparation tels qu'on les a décrits dans les cellules isolées.

A. Une dégradation du DNA : en prémarquant à l'aide de ^3H -TTP le DNA d'une suspension de mitochondries, on constate que l'irradiation de celle-ci produit une perte accélérée de radioactivité, fonction de la dose d'irradiation (Fig.1). En présence d'EDTA, inhibiteur des nucléases, la dégradation ne s'observe plus, tant dans les suspensions contrôlées que dans les suspensions irradiées.

B. Une hausse de l'incorporation des précurseurs : incubées en présence de 1 μmole d' ^3H -TTP des suspensions de mitochondries irradiées par 5kR incorporent plus de précurseurs que des suspensions témoins. Cette augmentation radio-induite d'incorporation est supprimée par l'addition de bromure d'éthidium, un inhibiteur de la DNA-polymérase mitochondriale, ou d'EDTA, un inhibiteur de la ligase et des nucléases (Fig. 2).

Signalons une communication récente de Clayton et al. qui décrit un processus d'excision de dimères de thymine dans des mitochondries irradiées in vitro par UV.

A. SHIMA, L. BAUGNET-MAHIEU and R. GOUTIER

On the increased incorporation into the DNA of rat liver mitochondria irradiated in vitro.

^xBoursier japonais du Service d'Echange culturel (Ministère belge de l'Education Nationale).

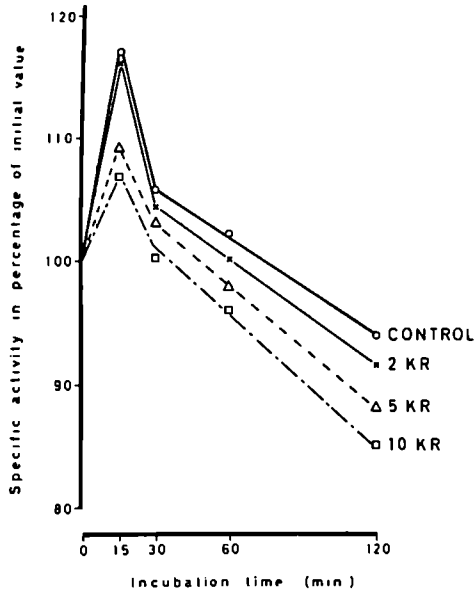


Fig. 1. Dégradation radio-induite du mt-DNA prémarqué par $^3\text{H-TTP}$, en tampon tris.

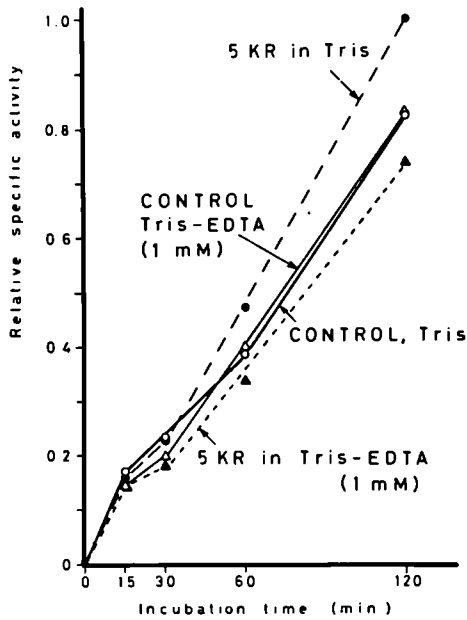


Fig. 2. Abolition, par l'EDTA, de la hausse radio-induite d'incorporation d' $^3\text{H-TTP}$ dans mt-DNA.

A remarquer que l'EDTA n'affecte pas le taux d'incorporation dans les mitochondries non irradiées.

Résultats du projet n° 3

Chef du projet et collaborateurs scientifiques :

J.R. MAISIN, G. MATTELIN, M. LAMBIET-COLLIER, C. BIESEMANS-VAN GENECHTEN.

Titre du projet : INFLUENCE DES RADIOPROTECTEURS SUR LES EFFETS A LONG TERME DES RADIATIONS IONISANTES.

Ces expériences sont réalisées principalement sur des souris mâles de race BALB/c⁺ âgés de 12 semaines, et secondairement sur des souris de races C57B1 et AKR. Comme radioprotecteurs nous utilisons l'AET (2-β-aminoethylisothiouronium-Br-HBr) la 5-hydroxytryptamine et une association d'AET de glutation de 5-Hydroxytryptamine, de cystéine et de mercaptoéthylamine.

D'après le traitement appliqué les souris sont exposées sur l'ensemble du corps à une dose unique ou multiple de radiations allant de 100 à 2000 R de rayons X. L'ensemble de ces expériences toujours en cours portent sur plus de 9000 souris.

Voici brièvement résumé les résultats obtenus à ce jour :

I. Survie

1. Les substances radioprotectrices protègent efficacement les souris contre le raccourcissement de la vie ;
2. La protection obtenue est moins marquée que pour les effets aigus des radiations ;
3. Le facteur de réduction de dose obtenu varie en fonction de la dose de radiation administrée. Exemple : après une exposition à une dose unique comprise entre 500 et 1200 R de rayons X l'association de substances radioprotectrices utilisées offre un facteur de réduction de dose d'environ 2. La protection obtenue est plus faible pour des doses moins et plus élevées de radiation. Rappelons que cette même association de substance radioprotectrice offre pour les effets aigus des radiations un facteur de réduction de dose égal 3.

II. Incidence des leucémies et des cancers

- A. Lorsqu'on compare des groupes des souris protégées ou non et exposées à une même dose de radiation, on constate que :

1. L'incidence des leucémies lymphoïdes et surtout thymiques est sensiblement plus faible chez les souris protégées que chez les souris non protégées.
 2. Pour les faibles doses de radiations (350 R et inférieure) l'incidence des leucémies myéloïdes est plus faible chez les souris protégées par une association de substances radioprotectrices que chez les souris non protégées.
 3. Pour l'ensemble des leucémies et des cancers, la période de latence avant l'apparition des cancers est en général plus longue pour les souris protégées et irradiées que pour les souris non protégées.
- B. Lorsqu'on compare des groupes de souris ayant la même survie médiane, le taux des leucémies et des cancers est d'environ deux fois moindre chez les souris protégées que chez les souris non protégées. Exemple : les souris non protégées et irradiées avec 500 R présentent deux fois plus de leucémies et de cancers que les souris protégées et irradiées avec 1000 R de rayons X.
- C. Par quels mécanismes d'actions des radioprotecteurs peuvent-ils influencer le taux des leucémies et des cancers ?
 Ces recherches se poursuivent activement. Les radioprotecteurs semblent agir à la fois sur les effets déclencheurs et sur les effets promoteurs des radiations sur le processus de carcinogénèse. Les effets déclencheurs concernent les effets immédiats survenant au cours de l'interaction des radiations avec les macromolécules cellulaires. Dans ce cadre les radioprotecteurs semblent agir entre autres sur la libération des virus oncogènes et en protégeant les cellules cibles. Parmi les effets promoteurs des radiations, mentionnons : la diminution de la défense immunologique. Or, il est certain que les radioprotecteurs peuvent agir en protégeant le système immunologique des animaux irradiés.

III. Effets non néoplasiques des radiations

L'association de substances radioprotectrices utilisées protège efficacement les souris irradiées contre l'augmentation de l'incidence des gloméruloscléroses. Par contre elle semble n'avoir qu'un faible effet protecteur sur les lésions pulmonaires.

J.R. MAISIN, A. DECLEVE, M. LAMBIET-COLLIER and G. MATTELIN

Late effects of radiation and chemical protectors.
Abstracts of papers for the Twentieth Meeting of the Radiation
Research Society. Portland, Oregon, May 14-18, 1972.

G.B. GERBER, A. DECLEVE, J.R. MAISIN, A. LEONARD and Gh. MATTELIN

Fate of DNA labeled bone marrow cells from different mouse strains
injected into syngeneic irradiated recipients.
Experientia : 28, 149-151, 1972.

A. DECLEVE, G.B. GERBER, A. LEONARD, M. LAMBIET-COLLIER, A. SASSEN, J.R. MAISIN

Regeneration pattern of thymus, spleen and bone marrow in X-irradiated
AKR mice with or without syngeneic bone marrow transplantation.
Rad. Research : 51, 318-331, 1972.

Résultats du projet n° 4

Chef du projet et collaborateurs scientifiques :

A. LEONARD, B. IVANOV, Gh. DEKNUDT

Titre du projet : ETUDE DES REMANIEMENTS CHROMOSOMIQUES PRODUITS PAR LES RAYONS-X CHEZ LA SOURIS MALE.

Nous avons montré précédemment que la réaction primaire à l'irradiation des cellules reproductrices mâles et notamment des spermatogonies pouvait être perturbée par des phénomènes secondaires tels que l'élimination sélective des cellules les plus lésées. Afin d'approfondir la question nous nous sommes d'une part intéressés à ce qui se passe chez les individus mâles irradiés in utero et d'autre part à l'effet radioprotecteur éventuel que pourrait avoir l'administration de divers composés chimiques avant l'irradiation.

A. L'effet radioprotecteur d'un mélange de glutathion (GSH), 2-β-aminoethylisothiocourée-Br-HBr (AET), mercaptoéthylamine (MEA), cystéine et sérotine, vis-à-vis des remaniements chromosomiques produits par les radiations ionisantes a été étudié chez la souris. Dans ce but nous avons observé chez les animaux traités les spermatocytes en division. Nos résultats (Tableau) montrent que l'administration du mélange avant une exposition aux radiations ionisantes diminue le taux de translocations produites par les radiations. De nos résultats on peut donc conclure que l'utilisation de ce mélange ne permet pas la survie de cellules lésées mais protège réellement les spermatogonies contre l'induction des translocations.

Observations	Traitement			
	Témoins	Témoins + mélange	500 R	Mélange + 500 R
Souris examinées	10	14	20	9
Cellules analysées	2000	2750	3980	1800
Métaphases anormales	2	2	321	107
%	0,1	0,07	8,07	5,94

B. Des souris femelles gravides ont été irradiées sur tout le corps avec des doses de 0, 100, 200 ou 300 R de rayons X au 13 1/2 jours de la gestation. Nous avons choisi ce moment parce que les cordons sexuels sont en train de se différencier et que les gonocytes se divisent activement. La mortalité prénatale n'a pas été modifiée mais par contre la mortalité postnatale augmente avec la dose d'irradiation (Tableau). Les souris mâles irradiés in utéro ont été sacrifiés afin de déceler dans les spermatoocytes en division les remaniements chromosomiques éventuels. Comme nous n'avons observé aucune anomalie chromosomique il semble donc qu'il existe entre les gonocytes et les spermatogonies A de l'adulte une grande différence de radiosensibilité. Chez ce dernier type de cellule en effet le taux d'anomalies augmente linéairement avec la dose d'irradiation.

SURVIE DES DESCENDANTS IRRADIÉS IN UTERO

Traite- ment R	Nombre de femelles traitées	Nombre de des- cendants à la naissance		Nombre de descendants au sevrage				Perte entre la naissance et le sevrage (%)
		Total	Moyenne ± S.E.	Total	Moyenne ± S.E.	♂	♀	
0	10	61	6.1 ± 0.37	61	6.10 ± 0.37	32	29	0
100	9	63	7.0 ± 0.52	59	6.55 ± 0.64	27	32	6.3
200	23	140	6.9 ± 0.42	126	5.47 ± 0.76	64	62	10.0
300	18	100	5.5 ± 0.34	72	4.00 ± 0.40	37	35	28.0

- A. LEONARD, Gh. DEKNUDT, G. LINDEN et N. GILLIAVOD
Strain variations in the incidence of dominant lethals induced by X-irradiation given to mouse spermatozoa.
Strahlentherapie : 143, 102-105, 1972.
- A. LEONARD et N. GILLIAVOD
Radiosensitivity of male germ cells in rats and mice.
Meeting of Belgian and Netherlands Radiobiological Society, Abstract.
- A. LEONARD et GH DEKNUDT
Effect of AET on chromosome rearrangement induced by X-irradiation in spermatogonia.
Radiation Research 50, 120-124, 1972.
- A. LEONARD
Données récentes sur les taux de mutations radio-induites chez les mammifères.
4th Internat. Congress of Human Genetics, 241-252, 1972.
- N. GILLIAVOD et A. LEONARD
Etudes des réarrangements chromosomiques produits dans les spermatogonies du rat et de la souris par une exposition aux rayons-X.
Eur. J. Genet. Cytol. 14, 341-345, 1972.
- A. LEONARD et Gh. DEKNUDT
Chemical radioprotection against chromosome rearrangements induced in spermatogonia of mice.
ESRB Meeting, Abstract.
- N. GILLIAVOD et A. LEONARD
Radiosensibilité comparée des rats et souris mâles.
C.R.Soc. Biol. 116, 209-211, 1972.

Résultats projet n° 5

Chef du projet et collaborateurs scientifiques :

A. LEONARD , N. GILLIAVOD.

Titre du projet : Etude des remaniements chromosomiques produits par une irradiation des cellules reproductrices femelles.

Des souris femelles de race C57Bl ont été irradiées avec 0, 50 ou 200 R de rayons X et ont été croisées avec des mâles témoins de la même race. Le tableau ci-joint montre que toutes les femelles témoins ont donné plus de 3 nichées alors que la plupart des animaux irradiés n'ont donné qu'une seule nichée. Nous avons examiné les testicules de 100 mâles témoins et de 185 mâles descendants des femelles irradiées. Chez aucun nous n'avons observé la présence de translocations.

Afin de faire la part exacte d'un processus éventuel de sélection germinale nous avons examiné les chromosomes méiotiques des femelles 24 heures après une exposition à ces mêmes doses de 50 ou 200 R. La technique utilisée a consisté à incuber les oocytes dans du sérum fœtal de veau pendant 4 heures à 37°C. Les résultats des observations effectuées jusqu'à présent sont résumés dans le tableau ci-contre.

Les observations effectuées sur les chromosomes méiotiques des femelles irradiées ne sont encore que partielles. Sans qu'il soit possible à l'heure actuelle d'établir une relation entre la dose de rayons X administrée et le taux d'anomalies observées il est cependant certain que les radiations ionisantes peuvent produire des translocations dans les oocytes.

Résultats de l'observation des oocytes

Traitement	Oocytes examinés	Oocytes animaux
0 R	50	0
50 R	50	2 (CIV-RIV)
200 R	50	0

Analyse de la fertilité des souris témoins et des souris irradiées in utero

Traitement	Nombre de femelles	Femelles avec au moins une nichée		Femelles avec au moins 2 nichées		Femelles avec au moins 3 nichées	
		Nombre	Nombre moyen de jeunes par nichée	Nombre	Nombre moyen de jeunes par nichée	Nombre	Nombre moyen de jeunes par nichée
0 R	25	25	5.08	25	5.88	25	5.88
50 R	25	21	6.24	9	5.78	4	4.00
200 R	25	23	6.22	8	5.38	-	-

Résultats du projet n° 6

Chef du projet et collaborateurs scientifiques :

A. LEONARD , Gh. DEKNUDT.

Titre du projet : Etude des anomalies chromosomiques produites par les irradiations, au moyen des techniques de fluorescence et de "banding pattern".

Les recherches effectuées jusqu'à présent comportaient 2 volets. Dans un premier travail nous avons produit des individus transloqués par irradiation des stades postméiotiques de la souris mâle. Quelques cents descendants ont été ainsi produits parmi lesquels 25% environ étaient porteurs de translocations ainsi que l'a montré l'observation des chromosomes méiotiques effectués sur un testicule. Les animaux porteurs de translocations ont été maintenus en vie afin de permettre l'observation ultérieure de leurs chromosomes somatiques par les techniques de fluorescence et de "banding pattern".

Sur des individus possédant des chromosomes marqueurs (AKR/T1Ald) nous avons commencé la mise au point des techniques de préparation. Durant de nombreux mois nous nous sommes heurtés à de nombreux problèmes pratiques résultant notamment de la difficulté à se procurer les produits chimiques indispensables. Actuellement nous sommes à même d'obtenir quelques 5 à 10% de cellules parfaitement analysables. Divers appareils susceptibles de traduire graphiquement les différences de densité obtenues ont été testés et une de ces installations sera acquise en 1973.

Les individus transloqués produits dans le premier volet de ce travail seront alors systématiquement analysés afin d'établir les relations entre les figures de translocations observées dans les spermatoocytes en division et les modifications de banding pattern obtenues dans les chromosomes des cellules somatiques.

Vertrag Nr. 090-72-1 BIAD

Institut für Biologie der Gesellschaft für Strahlen- und
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K.-H.Marquart, W.A. Müller, J.Murken, F.Schales, H.Spiess,
W. Wiederholt, W.A. Winter.

Pathogenesis of radiation damage.

Radiotoxicity

After incorporation of bonesseeking radionuclides in rodents the nature and development of early and late effects, especially the production of bone tumors is studied. The dependency upon the mean skeletal dose and the role of dose distribution in time and space are investigated by using various radionuclides differing by the radiation emitted, their halflives and their mode of deposition in bone (volume- and surface seeker).

Distribution studies were extended to some rare earths with regard to later application in longterm experiments. Two automatic methods for quantitative evaluation of α -autoradiograms can now be used.

As compared to single injection the repeated injection of ^{224}Ra (half-life 3.64 days) corresponding to the same total skeletal dose resulted in a significant higher osteosarcoma incidence. A similar high osteosarcoma risk has been observed after single injections of the longer lived ^{227}Th . (half-life 18.7 days).

Histogenesis, classification and nomenclature of spontaneous and experimentally induced bone tumors and liver tumors are subject of study in contact with EULEP.

Besides late effects early radiation effects on the ultrastructure of osteocytes and the cellular composition of osteogenic tissue are studied.

The isoproterenol-stimulated salivary gland is used for investigation of the radiation sensitivity of an induced cell cycle.

Two epidemiological studies are concerned with the late effects of ^{224}Ra after therapeutic application in juveniles and in patients with ankylosing spondylitis.

Project 1

Late effects after incorporation of short-lived bone seeking radionuclides
W. Gössner, I. Günzel, O. Hug, A. Luz, W.A. Müller

Distribution studies and dosimetry were carried out with the short-lived β -emitters ^{140}La , ^{142}Pr and ^{147}Nd .

An experiment with ^{141}Ce has been started using 1000 mice and injected activities of 100 - 10,000 $\mu\text{Ci}/\text{kg}$. Depending on the injected amount of inactive Cerium, which was present in the solution as a carrier, the distribution of radioactivity changed within the body. Thus in the highest ranges of activities applied the dose of liver reaches values several times higher than that of skeleton. This fact complicates the intended application of Cerium for quantitative studies of late effects.

Incorporation of ^{90}Sr in male mice showed a strong dependency of soft tissue dose burden on initial ^{90}Y content in the injection solution.

In addition to quantitative autoradiography by track counting we started experiments with photometric measurements in incident light of unstained autoradiograms. Within a wide range there is a clear linear relationship of the number of α -tracks with the photometric data.

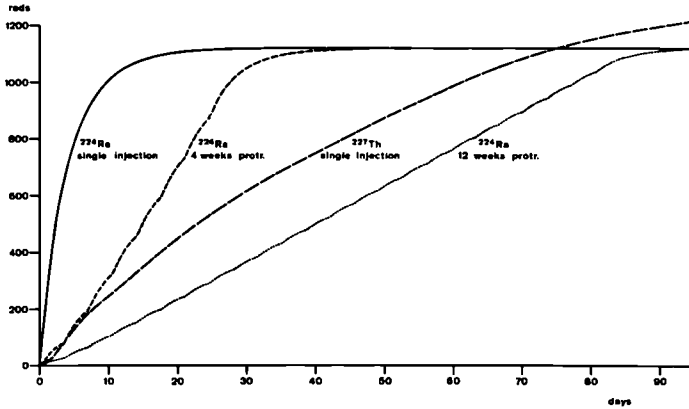
The automatic counting of α -tracks done by holographic filtering of direction combined with a picture analysing method (classimat) can now be used for our experimental material.

The osteosarcoma incidence of the whole skeleton after single injections of ^{224}Ra (25 $\mu\text{Ci}/\text{kg}$) in young (4 weeks) and adult (5 - 6 months) female NMRI-mice shows no distinct difference. But the fraction of head tumors was somewhat higher in the adult animals.

With protracted application of ^{224}Ra an increased tumor incidence has been observed as compared to single injections producing the same skeletal dose. For instance after repeated injection of 1.5 μCi $^{224}\text{Ra}/\text{kg}$

(3.5 days intervall) over 12 weeks, corresponding to 36 $\mu\text{Ci}/\text{kg}$ or 1100 rads mean skeletal dose, the incidence of non-head osteosarcomas reached a value of 60 % within 21 months or 80 % (data corrected for mortality). The corresponding values after single injection were 8 % and 15 % respectively.

The osteosarcoma incidence after single injection of the longer lived ^{227}Th (half-life 18.7 days) reaches nearly the values observed after repeated application of ^{224}Ra producing the same skeletal dose. This also indicates the role of time distribution of dose for bone tumor induction (compare figure 1 a and 1 b).



a) Fig. 1 a

Accumulation of skeletal doses in mice after single and repeated injections of ^{224}Ra and ^{227}Th up to a final dose of about 1100 rads.

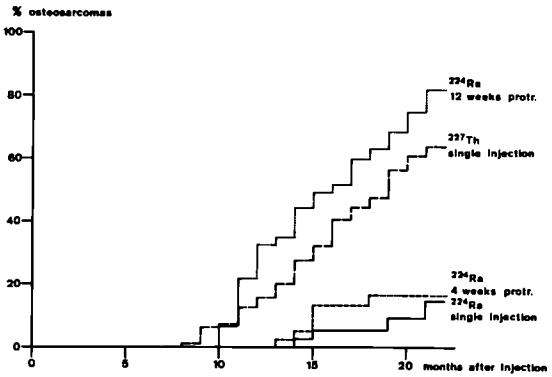
Mode of application

^{224}Ra single injection: $36 \mu\text{Ci/kg}$

^{227}Th single injection: $5 \mu\text{Ci/kg}$

^{224}Ra $36 \mu\text{Ci/kg}$ protracted over 4 weeks: $4.5 \mu\text{Ci/kg}$, 8 times, with 3, 5 days intervall

^{224}Ra $36 \mu\text{Ci/kg}$ protracted over 12 weeks: $1.5 \mu\text{Ci/kg}$, 24 times, with 3, 5 days intervall



b) Fig. 1 b

The monthly progressing incidence of non-head osteosarcomas after single injection of ^{227}Th and ^{224}Ra and protracted application (4 weeks and 12 weeks) of ^{224}Ra . In all experimental groups the mean skeletal dose is about 1100 rads. (The data are corrected for mortality.)

Project 2

Histogenesis, classification and nomenclature of radiation induced tumors.

W. Gössner, A. Luz

The second slide seminar of the committee on pathology standardization of the EULEP has been organized in Munich in June 1972.

The classification of bone tumors in experimental animals has been discussed again. As the result of the work of the committee a picture atlas of bone tumors is now ready for press. The main topic of the slide seminar 1972 was liver tumors in experimental animals (mice, rats, rabbits).

On behalf of the International Agency for Research on Cancer (WHO) a chapter on tumors of the jaws in mice has been prepared for the Manual on the Pathology of Tumors in Laboratory Animals.

Project 3

Pathogenesis of early effects

A. The effects of ^{224}Ra and ^{227}Th on the cellularity of osteogenic tissue

G. Becker.

The number of osteoblasts and mesenchymal cells per unit area in the tibia metaphysis decreases at higher doses of ^{224}Ra and ^{227}Th . There might be a causal connection with the decrease of tumor incidence in the same dose range.

After $5 \mu\text{Ci } ^{227}\text{Th}/\text{kg}$ however the loss of cells in the lumbar vertebra is somewhat higher than in the tibia metaphysis in spite of the higher tumor risk of a single lumbar vertebra.

B. Electron microscopic study of the young osteocytes in the tibia of mice after incorporation of ^{224}Ra .

K. -H. Marquart

The ultrastructure of the young ("osteoblast-like") osteocytes in the proximal tibial metaphysis of mice was studied 2, 24 hours and 5 days after incorporation of 1, 1.5 and $5 \mu\text{Ci } ^{224}\text{Ra}/\text{kg}$.

Even 2 hours after incorporation more than half of the cells examined show ultrastructural alterations of nuclei, mitochondria, endoplasmic reticulum and Golgi apparatus.

The mitochondria appeared to be the most sensitive of the cellular organelles.

C. Irradiation effect on the isoproterenol-stimulated cellproliferation in the salivary glands of mice.

W.A. Winter

Single irradiation with different doses between 100 rad and 800 rad at different times during the whole presynthetic-phase supresses the isoproterenol induced DNA-synthesis in the salivary glands of mice. The irradiation effect was observed even in the resting cells up to 48 hours before injection of isoproterenol. These experiments were done with liquid scintillation technique.

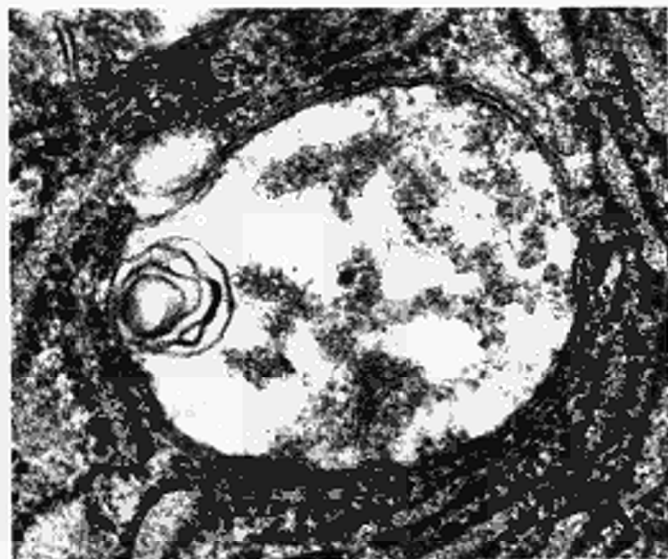


Fig. 2

Electron microscopic picture of a mitochondrion of a young ("osteoblast-like") osteocyte 24 hours after incorporation of $5 \mu\text{Ci } ^{224}\text{Ra/kg}$. The mitochondrion shows swelling, loss of cristae and an intramitochondrial myelin-like figure.

Project 4

Studies on late effects induced by ^{224}Ra in children and adults.

H.Spiess, A.Gerlach, J. Murken, W. Wiederholt

This project, which was supported in the last years by the foregoing EURATOM-Associationcontract has been continued. Additional 475 persons which received for therapeutic purpose Ra-224 as adults, have been checked up by questionnaires and/or reports of family doctors. 35 cases of death were registered amongst which 5 soft-tissue cancers has been found. The statistical analysis of the data is in progress.

Project 5

Epidemiological study on late effects after medical application of ^{224}Ra in ankylosing spondylitis patients.

O.Hug, F. Schales

From 1949 up to now about 2000 patients suffering from ankylosing spondylitis were treated in several German orthopedical hospitals with ^{224}Ra .

In 1971 additional to project 4 a study on possible radiation effects in this group was started. The aim of the study is to calculate the average skeletal radiation doses received by these patients and to correlate to them late effects to be found by clinical re-investigations.

Up to now contracts were concluded with 3 hospitals in Frankfurt/Main, Münster and Hannover. Other hospitals have been or will be contacted.

In Frankfurt 357 patients are available, 152 of them have been re-investigated. 24 out of the ^{224}Ra group and 24 of a control group have died. The causes of death have been recorded. Nearly all of the remaining patients answered a questionnaire.

The average skeletal doses range from 10 to about 200 rad with the bulk in the 50 - 60 rad region.

In Münster about 1 550 names of spondylitis patients were found. Their case reports are being scanned for ^{224}Ra treatment. About 110 questionnaires have been answered up to now; the re-investigations will start in 1973.

In Hannover about 200 patients were found out, about 20 of them being re-investigated in 1972.

The collection and evaluation of patient data by the help of EDP will be done parallel to project 4 and was prepared in agreement with the EURATOM thorotrast group in Heidelberg.

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Epidemiologische Studie über Strahlenschäden nach der therapeutischen Anwendung von Thorium X (Ra-224). Tagung der Deutschen Gesellschaft für Biophysik, Erlangen, 4.-6.10.1972 (Diskussionsbemerkung)

Schales, F.

Strahlenschäden durch Thorium X (Ra-224)
Biophysikalisches Kolloquium der Universität Homburg/Saar, 29.11.1972

Vertrag Nr. o63-72-o1 PST D

Institution: Institut für Nuklearmedizin des Deutschen Krebsforschungszentrums, Heidelberg (DKFZ).

Projektleitung: Prof.Dr.K.E.Scheer, Direktor des Instituts für Nuklearmedizin (IfN) des DKFZ.

Stellvertreter: Priv.Doz.Dr.W.J.Lorenz, IfN

Koordinator: Dr.G.van Kaick, IfN

Das Vertragsprogramm wird in Zusammenarbeit mit

Prof.Dr.H.Muth, Direktor des Instituts für Biophysik der Universität des Saarlandes (Boris Rajewski-Institut), Homburg

Prof.Dr.G.Wagner, Direktor des Instituts für Dokumentation, Information und Statistik des DKFZ und

Prof.Dr.A.Kaul, Nuklearmedizinische Abteilung, Klinikum Steglitz der Freien Universität Berlin,

durchgeführt.

Thema: Forschungsvorhaben "Thorotrast" - Untersuchungen zur Beurteilung der durch künstliche Bestrahlung bewirkten Spätschäden beim Menschen (Thorotrast-Patienten).

Allgemeine Darstellung der durchgeführten Arbeiten:

Die Ziele des Forschungsvorhabens wurden vom Koordinierungsausschuß, dem Vertreter des Bundesministeriums für Forschung und Technologie, von EURATOM, des DKFZ und des Instituts für Biophysik Homburg angehören, gemeinsam erarbeitet und festgelegt. Das vom Bundesministerium für Forschung und Technologie sowie von EURATOM unterstützte Forschungsvorhaben "Thorotrast" hat zur Aufgabe:

1. Biophysikalische und klinische Untersuchungen von Thorotrast-Trägern und von Patienten einer Kontrollgruppe.
2. Aufklärung der Spätschicksale bereits verstorbener Patienten der Thorotrast- und Kontrollgruppe.
3. Einsatz einer kombinierten klinischen Diagnostik zur Feststellung thorotrastinduzierter Neoplasien.

4. Follow-up-Untersuchungen von Patienten der Thorotrast- und der Kontrollgruppe sowie Klärung der Todesursache in zwischen verstorbenen Patienten.
5. Statistische Auswertung der Untersuchungsergebnisse.
6. Bestimmung der Chromosomenaberrationsrate bei Thorotrast-Trägern und Vergleichspersonen und ihre Abhängigkeit von der Strahlendosis.
7. Experimentelle Untersuchungen zur Analyse des Fremdkörperreizes und der Strahlenwirkung von Thoriumdioxid-Konglomeraten.

Ergebnisse des Projekts Nr. 1: Arbeitsgruppe Institut für Nuklearmedizin des Deutschen Krebsforschungszentrums Heidelberg (DKFZ) in Zusammenarbeit mit dem Institut für Dokumentation, Information und Statistik des DKFZ.

Leiter des Projekts und wissenschaftliche Mitarbeiter:

1. Prof.Dr.K.E.Scheer (Vertragsnehmer), PD Dr.W.J.Lorenz (Stellvertreter)

Dr. G. van Kaick (Koordinator)

Wissenschaftler des IfN: Dr. I. Drings (ab 1.10.72)

PD Dr. P. Georgi

Dr. U. Herzfeld

Dr. H. Kampmann

Dr. D. Lorenz

Dr. T. Oeftering (bis 30.9.72)

Dr. P. Schmidlin

Statistische Auswertung: Prof. Dr. G. Wagner

Prof. Dr. H. Innich

Titel a:

Recherchen nach Thorotrastpatienten und Patienten der Kontrollgruppe; Aufklärung der Spätschicksale verstorbener Patienten.

Titel b:

Klinische und biophysikalische Untersuchungen von Thorotrastpatienten und Patienten der Kontrollgruppe.

Titel c:

Diagnostik thorotrastinduzierter Hepatopathien und Neoplasien.

Titel d:

Follow-up-Studie: Nachuntersuchung der Thorotrastpatienten und der Patienten aus der Kontrollgruppe in 2jährigen Abständen sowie Ermittlung der Todesursachen zwischenzeitlich verstorbener Patienten.

Darstellung der Ergebnisse:

zu a): Recherchen wurden im Jahr 1972 an 20 verschiedenen Kliniken in der BRD und in Westberlin durchgeführt. Dabei konnten im Berichtszeitraum 400 Patienten erfaßt werden, denen vor Jahrzehnten im Rahmen einer Angiographie sehr wahrscheinlich Thorotrast inkorporiert worden war. Gleichzeitig wurden für die Kontrollgruppe etwa 800 Patienten aus denselben Kliniken erhoben. Die Gesamtzahlen erhöhen sich damit auf 6500 vermutliche Thorotrast-Träger und 14000 Vergleichspatienten.

Spätschicksale verstorbener Patienten: von 1200 Patienten der Thorotrastgruppe und 800 Patienten der Kontrollgruppe, die später als 3 Jahre nach dem damaligen Klinikaufenthalt verstorben sind, konnte die Todesursache aufgeklärt werden. Vergleichsweise ist der Anteil der primären Lebertumoren, der Leukämien, der Panmyelophthisen und der Leberzirrhosen in der Thorotrastgruppe wesentlich höher als in der Kontrollgruppe. Die Zahl der Lungentumoren ist in beiden Gruppen etwa gleich groß.

Todesursachen verstorbener Thorotrast-Träger (1200) im Vergleich zu einer Kontrollgruppe (800)

	Thorotrast- gruppe	Kontroll- gruppe
Primäre Lebertumoren	65	7
Leberzirrhosen, Leber- dystrophien	70	20
Leukämien	17	1
Myelophthisen	12	1
Lungentumoren	22	18

zu b): Im Rahmen der Thorotrast-Studie wurden bisher in Heidelberg 1300 Patienten aus allen Teilen der Bundesrepublik und des benachbarten Auslandes untersucht. Im Berichtsjahr betrug die Zahl der Untersuchungen insgesamt 279:

87 Patienten wurden erstmals als Thorotrast-Träger entdeckt; bei 21 weiteren Patienten bestand zwar aufgrund der Eintragungen in den alten Krankenblättern der Verdacht auf eine Thorotrastapplikation; es konnten jedoch keine Thorotrastablagerungen nachgewiesen werden. Die 64 erstmals untersuchten Patienten der Kontrollgruppe kamen vorwiegend aus von Heidelberg weit entfernten Städten. Im Berichtsjahr wurde mit der Wiederuntersuchung bereits bekannter Thorotrast- und Kontrollpatienten begonnen; dabei wurden 107 Patienten erneut untersucht. (siehe Titel d)

zu c): Für die Diagnostik thorotrastinduzierter Neoplasien und Hepatopathien kamen routinemäßig Laboruntersuchungen, Bestimmung des Alpha-1-Foetoproteins, Röntgenaufnahmen der Lunge und des Abdomens sowie eine sonographische Darstellung der Leber zum Einsatz. Bei klinischer Indikation wurde außerdem eine Leberszintigraphie innerhalb der ambulanten Diagnostik angeschlossen. Ergaben sich daraus Hinweise für eine fortgeschrittene Hepatopathie oder ein Neoplasma der Leber, so wurde im Rahmen einer stationären Diagnostik eine Leberangiographie bzw. eine Laparoskopie mit gezielter Probeentnahme aus der Leber empfohlen bzw. in einer Heidelberger Klinik durchgeführt. Bei 80 Thorotrast-Trägern konnten Hepatopathien leichteren bis mittleren Grades entdeckt werden; bei 17 weiteren Patienten bestanden schwerwiegende degenerative Veränderungen, wobei in einigen Fällen eine stationäre Diagnostik erforderlich wurde. - Die Ultraschalldiagnostik der Leber hat sich in Ergänzung zur Szintigraphie bewährt. Sie ist besonders für die Wiederholungsuntersuchung von Thorotrast-Trägern von Vorteil, da sie keine weitere Strahlenbelastung des Organs mit sich bringt.

zu d): Im Rahmen der Follow-up-Studie wurden jene Patienten erneut angeschrieben, die in den Jahren zwischen 1968 und 1970 erstmals von uns untersucht worden waren. Von allen

noch lebenden Patienten erhielten wir die Fragebogen beantwortet zurück. Im Jahr 1972 konnten 107 Patienten, d.h. 36 Thorotrastpatienten und 71 Kontrollpatienten wieder untersucht werden.

Etwa 10-15 % der angeschriebenen Patienten sind inzwischen verstorben. Die Ermittlung der Todesursache ergab eine weitere Zunahme an primären Lebertumoren und Leukämien: von 18 Patienten, deren zum Tode führende Erkrankung geklärt werden konnte, verstarben 6 Patienten an einem primären Lebertumor und 1 Patient an einer Leukämie. Diese wenigen Zahlen belegen die wesentliche Bedeutung der Follow-up-Studie, da mit zunehmender Latenzzeit wahrscheinlich mit einem gehäuftem Auftreten von thorotrastinduzierten Neoplasien zu rechnen ist.

Veröffentlichungen

van Kaick, G. und P. Drings:
Spätschäden des Knochenmarks nach intravasaler Thorotrastanwendung. Vortrag auf der 10. internationalen Jahrestagung der Gesellschaft für Nuklearmedizin vom 27.-30. September 1972 in Freiburg.

Projekt Nr. 2: Arbeitsgruppe Institut für Biophysik der Universität des Saarlandes, 665 Homburg (Saar).

Leiter des Projektes: Prof. Dr. H. Muth

Titel: a) Klinische und biophysikalische Untersuchungen an Thorotrastpatienten.

Wissenschaftliche Mitarbeiter: Dr. med. B. Schatanek
Dipl.-Phys. P. Schneider
Dr. rer. nat. W. Kemmer
Prof. Dr. H. Muth
Prof. Dr. Dr. E. Oberhausen

Titel: b) Untersuchungen von Chromosomenaberrationen bei Thorotrastpatienten.

Wissenschaftliche Mitarbeiter: Dr. W. Kemmer (Biologe)
Prof. Dr. H. Muth

Titel: c) Tierexperimentelle Untersuchungen in Zusammenarbeit mit den Arbeitsgruppen Prof. Dr. A. Kaul, Berlin, und der Arbeitsgruppe Heidelberg.

Wissenschaftliche Mitarbeiter: Dr. W. Kemmer
Prof. Dr. H. Muth

Darstellung der Ergebnisse:

zu a): Die bereits im Jahre 1971 vorbereiteten Untersuchungen eines speziellen Personenkreises, der mit hoher Wahrscheinlichkeit eine Thorotrastapplikation erhalten hatte, aber entweder aus gesundheitlichen oder familiären Gründen nicht die Untersuchungsstellen in Heidelberg oder Homburg (Saar) aufsuchen konnte (siehe Jahresbericht 1971) wurden im Jahre 1972 begonnen. Diese Patienten wurden mit dem institutseigenen Meßwagen aufgesucht. Jeweils an Ort und Stelle wurde die Thoronkonzentration der Atemluft mittels einer speziell entwickelten, transportablen Meßapparatur bestimmt. Diese Meßwerte gestatten - ähnlich wie die Messungen im stationären Ganzkörperzähler - eine Rückrechnung auf die ursprünglich injizierten

Thorotrastmengen. Auch die vorgesehenen klinischen Untersuchungen wurden durch einen ärztlichen Mitarbeiter in der Wohnung des Patienten durchgeführt. In allen Fällen war diese Untersuchung vorher mit dem zuständigen Hausarzt abgesprochen worden. Die labordiagnostischen und cytogenetischen Untersuchungen an den Blutproben der Patienten wurden in den Kliniken und im Institut in Homburg vorgenommen.

Zur Vorbereitung dieser Untersuchungsaktion mit dem Meßwagen waren 105 Patienten angeschrieben worden. 58 davon hatten ihr Einverständnis mitgeteilt. 46 wurden inzwischen untersucht. Hiervon waren 44 Thorotrastträger. Bei 2 Patienten fanden sich keine Thorotrastdepots. Zu diesen ambulant untersuchten Patienten kamen noch 5 stationär untersuchte Patienten, so daß die Gesamtzahl im Jahre 1972 etwa 63 betrug. Eine weitere Aktion dieser Art, die etwa noch 100 Patienten einschließen wird, ist in Vorbereitung.

Zu b): Bei 23 aus der Gesamtzahl der untersuchten Patienten, bei denen die Lymphozyten-Kulturen positiv verliefen, wurden Chromosomenanalysen vorgenommen, die in gleicher Weise wie bisher (siehe Bericht für das Jahr 1971) ausgewertet wurden. Für die Abhängigkeit der Chromosomenaberrationsrate von der Strahlendosis ergab sich der gleiche Verlauf, wie er in den vorangegangenen Untersuchungen gefunden worden war. Die bereits festgestellte ausgeprägte biologische Variabilität der Untersuchungsergebnisse wurde erneut bestätigt. Die unter c) beschriebenen, neu begonnenen tierexperimentellen Untersuchungen sollen einen Beitrag zur Klärung dieses Problems erbringen.

Zu c): Auf Grund der bisher von der Arbeitsgruppe Prof. Kaul erhaltenen Ergebnisse wurde zur Klärung einiger noch offener Fragestellungen eine Zusammenarbeit im Rahmen der folgenden tierexperimentellen Untersuchungen (Kaninchen, chinesische Hamster) begonnen:

1. Weitere Thorotrast-Verteilungsstudien.

Insbesondere soll in diesen Tierexperimenten die Verteilung von Thorotrast und seiner Folgeprodukte im Organismus in Ab-

hängigkeit von verschiedenen hohen applizierten Thorotrastmengen bestimmt werden.

2. Abhängigkeit der Chromosomenaberrationsraten von der jeweils applizierten Thorotrastmenge und der Verteilung des Thorotrasts in den für die Entstehung der Chromosomenaberrationen verantwortlichen kritischen Organen. Hierbei besteht vor allem auch die Möglichkeit, die Wirkung des Thorotrast in der ersten Phase nach Applikation zu untersuchen. Außerdem erwarten wir von diesen Studien weitere Aufschlüsse zum Problem der biologischen Variabilität der Chromosomen-Aberrationsraten.

3. Pathologische und hämatologische Untersuchungen dieser Versuchstiere.

Da aus den unter 1. genannten Untersuchungen Tiermaterial zur Verfügung steht, das mit verschiedenen hohen Thorotrastmengen behandelt wurde, bietet sich die zusätzliche hämatologische und pathologische Auswertung an, die in Zusammenarbeit mit der Arbeitsgruppe Heidelberg durchgeführt wird. Diese Auswertung erlaubt einen direkten Vergleich mit entsprechenden Untersuchungen an Patienten.

Die im Jahre 1969 begonnene Entwicklung einer Methode der automatischen Analyse von Chromosomenaberrationen, deren bisherige Ergebnisse im Bericht 1971 dargelegt wurden, wurde auf Beschluß des für das gesamte Forschungsvorhaben zuständigen Koordinierungsausschusses aus dem Thorotrastprogramm herausgenommen. Diese Arbeiten sollen in Anbetracht ihrer generellen Bedeutung in erweitertem Umfang in einem gesonderten Vorhaben ("Cytoscan") weitergeführt werden.

Veröffentlichungen:

- (1) MUTH, H., EDELMANN, L., GRILLMAIER, R., HERZFELD, U., HEYDER, J., KAUL, A., KOEPPE, P., KUNKEL, R., OBERHAUSEN, E., ROEDLER, H.D., WERNER, E.: Radiation Dose in Different Organs of Thorotrast Patients. Assessment of Radioactive Contamination in Man, Proceedings of a Symposium Stockholm 22.-26. November 1971, International Atomic Energy Agency, Vienna, 1972
- (2) KEMMER, W.: Chromosome and Blood Changes in Thorotrast Patients. Vortrag, IV. International Biophysics Congress Moskau 7.-14. August 1972, Abstracts Bd. III

Ergebnisse des Projekts Nr. 3. Arbeitsgruppe Klinikum Steglitz
der Freien Universität Berlin

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof.Dr.A.Kaul

Dr.W.Riedel

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Dr.B.Hindringer

Dr.W.Abmayer

Gesellschaft für Strahlen- und Umweltforschung mbH., München,
Abteilung für Allgemeine und Experimentelle Pathologie und
Institut für Strahlenschutz.

Titel: Kinetik der Verteilung von kolloidalem ThO_2 in Organen
des RES und Berechnung der Strahlendosen.

- a) Berechnung der α -Strahlendosis unter Berücksichtigung der Selbstabsorption durch Kongglomeration des ThO_2 -Kolloids im RES

A.Kaul, B.Hindringer, W.Abmayer

- b) Fremdkörperreiz und Strahlenwirkung

W.Riedel, B.Müller, A.Kaul

Darstellung der Ergebnisse:

Zu a):

Problemstellung

Nach Kenntnis der zeitlich-räumlichen Verteilung von Th^{232} und seinen Folgeprodukten sowie der zeitlichen Änderung der Aktivitätsverhältnisse zwischen den Thorium-Folgeprodukten wurde versucht, die zeitliche Änderung der ThO_2 -Teilchengröße in den Organen des RES quantitativ zu erfassen und die daraus resultierende Reduktion der α -Strahlendosis zu berechnen.

Methodik

Die Untersuchungen wurden an Kaninchen durchgeführt, denen jeweils 2 cm^3 einer kolloidalen ThO_2 -Suspension intravenös injiziert worden war. Zu verschiedenen Zeiten nach Thorotrast-Inkorporation (0,125-392 Tage) wurden die Tiere getötet und von Leber und Milz histologische Paraffinschnitte hergestellt. Von den ungefärbten Schnitten wurden im Dunkelfeld kontrastreiche Mikrofotos angefertigt und mit Hilfe des flying-spot-Abtastverfahrens

analysiert. Dabei werden die einzelnen Bilder nach einer Grauschwelle bewertet und mit einer lokalen Auflösung von 240 x 240 Bildpunkten (bits; 1 bit = $8,2/\mu\text{m}^2$) online in den Kernspeicher eines Rechners gegeben. Aus den ermittelten Flächengrößen der Konglomeratanschnitte wurden die Konglomeratvolumina unter der Annahme kugelsymmetrischer Strukturen berechnet.

Ergebnisse

Unter Berücksichtigung der Gleichgewichtsverhältnisse zwischen Th^{232} und seinen Folgeprodukten und der Reichweite von α -Teilchen in ThO_2 wurde für die Organe Leber und Milz das Verhältnis der Gesamtzahl der aus allen ThO_2 -Konglomeraten emittierten zu der Zahl der entstehenden α -Teilchen abhängig von dem zeitlich veränderlichen Größenspektrum der Konglomerate berechnet. Dabei ergab sich, daß die Selbstabsorption von α -Teilchen als Folge der Konglomerierung des ThO_2 -kolloids in der Leber während des Beobachtungszeitraums gering zunimmt und etwa 400 Tage nach Inkorporierung 30 % beträgt. In der Milz dagegen können bereits nach 100 Tagen nur noch 50 % der in den Konglomeraten entstehenden α -Teilchen austreten.

Schlußfolgerungen

Obwohl bei den bisherigen Berechnungen das Energiespektrum der aus den kugelsymmetrischen ThO_2 -Teilchen austretenden α -Teilchen noch unberücksichtigt blieb, läßt sich aus den Ergebnissen bereits ermesen, daß die Reduktion der Strahlenbelastung in Leber und Milz durch Selbstabsorption von α -Teilchen in der Größenordnung von 30 bzw. 50 % liegt. Die in der Milz beobachtete Dosisreduktion kann dafür mitverantwortlich sein, daß in diesem Organ weniger häufig maligne Veränderungen beobachtet werden als in der Leber.

Zu b):

Problemstellung

Über den Mechanismus bei der Tumorgenese nach Thorotrastapplikation können aus den bisherigen in der Literatur beschriebenen Untersuchungen keine eindeutigen Aussagen gemacht werden. Als ätiologische Faktoren sind die chemische Toxizität, die Radioaktivität sowie der Fremdkörperreiz in Betracht

zu ziehen. Chemisch scheint sich Thorotrast, zumindest was das ThO_2 betrifft, völlig indifferent zu verhalten, da keinerlei Vergiftungsfälle bei der Monazitaufbereitung beobachtet wurden. Die Frage nach dem Fremdkörperreiz als möglicher ätiologischer Faktor soll in Tierexperimenten angegangen werden.

Methodik

Es ist beabsichtigt, eine der im folgenden genannten kolloidalen Lösungen in jeweils eine Gruppe von Kaninchen intravenös zu injizieren:

- 1) $\text{ZrO}_2/\text{HfO}_2$ -aquadol ("Zirkonotrast"/"Hafniotrast")
- 2) $^{95}\text{Zr}/^{181}\text{Hf}$ markiertes "Zirkonotrast"/"Hafniotrast"
("Radiozirkonotrast"/"Radiohafniotrast")
- 3) mit ^{230}Th angereichertes Thorotrast und eventuell
- 4) Lösung von Dextrin, das im Thorotrast als Schutzkolloid fungiert.

Nach der Applikation sollen Exkretions- und Retentionsstudien sowie Verteilungsstudien der Kolloide in den Organen des RES durchgeführt werden, aus denen Aussagen über die Dosis gemacht und Vergleiche über die gegebenenfalls unterschiedliche Wirkung von inaktiven und radioaktiven Kolloiden gezogen werden können.

Zur Herstellung von Thorotrast geht man von ThO_2 -Solen aus, die man durch Peptisation von frisch bereitetem ThO_2 mit 0,2 n HCl erhält. Das ThO_2 gewinnt man durch thermische Zersetzung von $\text{Th}(\text{C}_2\text{O}_4)_2 \cdot 6\text{H}_2\text{O}$ in einem elektrischen Ofen bei 530°C . Die so erhaltenen Stammsolen wurden elektronenmikroskopisch hinsichtlich ihres Dispersitätsgrades untersucht. Es ergab sich Übereinstimmung mit dem heute nicht mehr erhältlichen Thorotrast. Nach Stabilisierung des Stammsols mit Gelbdextrin wurde sein pH-Wert auf 7,5 und seine Konzentration auf 24% w/v eingestellt und das Gemisch anschließend zwecks Sterilisierung ca. 30 Minuten bei 120°C erhitzt. Zur Gewinnung von $\text{ZrO}_2/\text{HfO}_2$ -aquadolen durch Peptisation wird frisch gefälltes Zirkonium-/Hafniumhydroxid mit conc. HCl auf pH-Werte zwischen 0,6 und 2 eingestellt und mindestens 24 h am Rückfluß gekocht. Eine Partikelgröße zwischen 3 und 20 nm erhielten wir weder bei dieser Methode noch bei der Kondensation von ZrOCl_2 nach dessen Hydrolyse, wobei der pH-Wert sukzessive durch Zugabe eines schwach basischen Austauschharzes erhöht wird. Läßt man die Erhöhung des pH-Wertes dagegen während einer Elektrodialyse erfolgen, sollen die Partikeldurchmesser im Bereich von 3 bis 7 nm liegen.

Veröffentlichungen:

Hindringer, B.; Abmayer, W.; Kaul, A.: Erfassung morphologischer Veränderungen von Thorotrast-Konglomeraten durch elektronische Bildanalyse. Verhandlungen der Jahrestagung der Deutschen Gesellschaft für Pathologie in Graz, 1972, S.452-457.

Kaul, A.; Abmayer, W.; Hindringer, B.: Reduktion der Strahlendosis durch Selbstabsorption von α - Teilchen in ThO_2 -Konglomeraten des RES . Biophysik, 1973, im Druck .

Contractant de la Commission : Commissariat à l'Energie
Atomique (France)

N° du contrat : 100-72-1 BIAF

Chef des Groupes de Recherche: Dr. J. LAFUMA

Thème général du contrat : Métabolisme et action toxique
des radioéléments.

Les travaux réalisés en 1972 peuvent être divisés
en deux groupes:

- le premier comprend les recherches qui font suite à celles effectuées depuis plusieurs années par l'Association CEA-EURATOM. Ces recherches sont orientées dans trois directions: métabolisme des radioéléments, action toxique des radioéléments sur l'épuration alvéolaire, recherche de nouvelles techniques de décontamination interne.
- le deuxième groupe comprend des études nouvelles qui sont orientées vers l'action toxique des aérosols radioactifs émetteurs α ou β . Ces études ne peuvent être menées à bien qu'en utilisant les connaissances et les résultats acquis depuis des années sur le métabolisme des aérosols radioactifs.

COMPOSITION DU COMITE DE GESTION

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Résultats du projet n° 1

Chof du projet : Dr. J.C. NENOT

Collaborateurs scientifiques : Melle M. MORIN

Titre du projet : Métabolisme et action toxique des transuraniens

I/ Etudes métaboliques.

En 1972, les études métaboliques ont porté sur le californium 252. Emetteur de particules α et de neutrons, cet élément va être de plus en plus utilisé. Son métabolisme est proche de celui de l'Américium 241 qui a, comme lui, la valence III, mais il a aussi des points communs avec celui du Plutonium 238. Deux faits le distinguent:

a/ diffusion rapide à partir de la porte d'entrée (intramusculaire). La migration est en 24 heures de 65% pour le Cf252, contre 12% pour le Pu238 et 9% pour l'Américium 241 lorsque les trois éléments sont injectés sous forme de nitrate et au même pH.

b/ charge osseuse importante liée à la rapidité de la diffusion. Cette diffusion rapide impose la mise en jeu très précoce de la thérapeutique par le DTPA lors d'une contamination accidentelle par cet élément.

II/ Action toxique des radioéléments inhalés.

Nous avons étudié l'action toxique des radioéléments émetteurs α inhalés sous diverses formes physico-chimiques.

- L'activité pulmonaire a été déposée en une seule séance d'inhalation.

- Nos expériences ont porté sur trois radioéléments: le Pu239, le Pu238 et l'Am241.

Nous avons utilisé les formes physico-chimiques suivantes dont la période d'élimination pulmonaire est: Oxyde de Pu239 (120 jours), nitrate de Pu239 (75 jours), nitrate de Pu238 (40 jours), nitrate d'Am241 (20 jours) nitrate d'Am241 avec traitement chronique au DTPA (6 jours).

- Les activités déposées dans l'alvéole ont varié de microcuries à quelques nanocuries par gramme de poumon. Les animaux sont sacrifiés lorsque leur état général ne permet plus la survie, l'autopsie complète est pratiquée ainsi que l'examen anatomo-pathologique des organes.

Ces expériences sont en cours mais nous pouvons déjà en tirer les conclusions suivantes:

1/ le temps de survie dépend de l'activité initiale inhalée et non de la période d'épuration alvéolaire.

2/ pour une même activité initiale le temps de survie est identique, que les animaux présentent ou non une tumeur pulmonaire,

3/ le temps de latence des tumeurs, la localisation et le type cellulaire ne semblent pas jusqu'ici dépendre de la nature physico-chimique du radioélément.

4/ Et, lorsque l'on compare nos résultats avec ceux obtenus par W. BAIR sur des chiens, on voit que les deux espèces font des cancers pour les mêmes activités inhalées initiales par gramme de poumon mais que les temps de latence des deux espèces sont dans le rapport de leur durée de vie.

Enfin, nous observons sur les animaux faiblement contaminés et ayant, de ce fait, une longue survie, des cancers extrapulmonaires en nombre anormal et de localisation variée.

Résultats du projet n° 2

Chef du projet : Dr. J. LAFUMA

Collaborateurs scientifiques : H. SCHORN
R. BATTI
W. SKUPINSKI

Titre du projet : Etude des facteurs qui conditionnent l'épuration pulmonaire.

I/ Action toxique des émetteurs β sur l'épuration alvéolaire.

Nous avons fait inhaler à des rats de l'oxyde de Ce 141. Emetteur β d'énergie 430 kev, ce radioélément est produit par activation neutronique.

Les activités retenues par l'alvéole ont été de 0.05 à 1.3 microcuries par gramme poumon.

En dessous de 0.3 microcurie par gramme, nous n'avons pas constaté de perturbation de l'épuration alvéolaire. Au-dessus de cette valeur, l'élimination alvéolaire est d'autant plus ralentie que la charge alvéolaire est plus élevée.

Si l'on compare ces résultats avec ceux obtenus après inhalation de $^{239}\text{PuO}_2$, on constate qu'on a le même effet pour des activités inhalées de même ordre.

Des recherches antérieures pratiquées avec des inhalations de nitrate de Cerium 144 et du nitrate de Plutonium 239 avaient montré qu'à activités égales le Plutonium 239 perturbait beaucoup plus l'épuration alvéolaire que le Cerium 144.

Les animaux utilisés pour ces expériences sont conservés pour étudier l'action toxique à long terme des émetteurs 8 inhalés.

II/ Recherches sur la décontamination pulmonaire.

Après avoir étudié sur l'animal vivant l'action de divers corticoïdes et d'un inhibiteur des corticoïdes sur l'épuration alvéolaire nous avons entrepris des études "in vitro". Ces études visent à vérifier l'hypothèse suivant laquelle ces produits agissent en modifiant la mobilité des macrophages alvéolaires.

Les premiers résultats obtenus confirment l'effet inhibiteur des corticoïdes sur la mobilité des macrophages. Ces expériences "in vitro" vont être poursuivies pour rechercher des molécules susceptibles d'accroître la mobilité de ces cellules, puis ces molécules seront injectées à l'animal vivant pour chercher à accélérer l'épuration alvéolaire. Elles permettront aussi d'étudier facilement les macrophages de nombreuses espèces animales.

Résultats du projet n° 3

Chef du projet : M. W. MULLER

Titre du projet : Recherches de nouvelles méthodes de décontamination interne.

I/ Etude sur le cryptateur.

1/ Recherche du cryptateur dans les excréments de rats traités.

Une analyse des urines et fécès a été entreprise pour extraire puis déterminer quantitativement le taux d'élimination du cryptateur.

2/ Dosage in vitro de différents cryptateurs.

Un dosage original est en cours de mise au point permettant la titration quantitative du cryptateur. Nous avons été amenés à appliquer cette technique à l'analyse d'autres substances de même type et il semble que les résultats soient aussi bons.

II/ Synthèse du Bleu de Prusse colloïdal.

A la suite de la publication de BOZOGZADEH A. et CATSCH A. "Evaluation of the effectiveness of colloïdal and insoluble ferri hexacyanoferrates (II) in removing internally deposited radiocesium" Arch. int. Pharmacol. ther 1972, 197, 1 - 175-188, une nouvelle synthèse du Bleu de Prusse colloïdal a été réalisée dans le but d'éliminer tous les sous-produits risquant d'être toxiques et de montrer l'efficacité supérieure de ce produit par rapport à ceux déjà connus.

Cette synthèse s'accompagne d'une analyse complète: chimique, physicochimique et biologique.

An experimental comparative study of the behaviour of ^{237}Np , ^{238}Pu , ^{239}Pu , ^{241}Am and ^{242}Cm in bone.
Health Physics, 22, 1972

An experimental removal of ^{144}Ce , ^{241}Am , ^{242}Cm , and ^{238}Pu from the rat skeleton. Health Physics, 23, (1972).

Metabolic and therapeutic study following administration to rats of ^{238}Pu nitrate. A comparison with ^{239}Pu .
Health Physics, 23, (1972)

Problèmes posés par l'utilisation des données d'excrétion urinaire pour l'évaluation de la charge corporelle.
A.I.E.A. - SM - 150/55 (1972).

An experimental research on the treatment of contamination by actinide solutions
IRPA, Budapest (1972)

Traitement chirurgical des plaies radiocontaminées. A propos de 6 cas rapportés dans la littérature.
Rapport CEA-BIB-203 (1972)

The behaviour of ^{237}Np in the rat.
- à paraître dans Health Physics -

Trois articles sur les contaminations par ^{252}Cf et leurs traitements.
en voie de publication dans Health Physics,
Radioprotection,
Comptes-Rendus de l'Académie des
Sciences.

Die Beeinflussung der Clearance-rate von inhalierten $^{59}\text{Fe}_2\text{O}_3$ partikeln durch metopiron
10^e symposium international à BAD GASTEIN (Autriche)
10-13 Janvier 1972

Duennschicht chromatographie von Tryptophan-Metaboliten
Z. Klin. Chem. 10, 174 (1972)

The effects of some steroid hormones on "Alveolar macrophage activity in rats"
Paris - 25/27 Septembre 1972

Associato della Commissione: ENEL - Ente Nazionale Energia Elettrica
n. del contratto: O62 - 72 - 1 - PSTI
Capo del gruppo di ricerca: Prof. Antonio Farulla

Tema generale del contratto: Effetti della prolungata esposizione a bassi livelli di radiazioni ionizzanti; ricerche morfologiche, citochimiche e citogenetiche sui linfociti circolanti di soggetti professionalmente esposti al rischio delle radiazioni ionizzanti in un impianto nucleare di potenza dell'ENEL.

Le ricerche citogenetiche in corso mirano a portare un contributo alla soluzione del problema degli effetti sull'organismo umano di una prolungata esposizione professionale a bassi livelli di radiazioni ionizzanti.

Nel corso del 1972 sono stati effettuati, nella centrale elettronucleare ENEL del Garigliano, 57 esami citogenetici cromosomiali a dipendenti professionalmente esposti. Ad analoghe indagini sono stati sottoposti nello stesso periodo 13 soggetti controllo, di sesso maschile, di età compresa tra 30 e 50 anni, clinicamente sani e non esposti per motivi professionali o diagnostico-terapeutico alle radiazioni.

Le ricerche citochimiche e morfologiche sono orientate: a) allo studio delle modificazioni metaboliche che precedono la attivazione blastica in coltura e l'inizio della sintesi del DNA, modificazioni che riflettono verosimilmente i cambiamenti necessari alla trasformazione della cromatina inattiva in un "template" attivo per la sintesi del DNA; b) alla identificazione, mediante l'analisi biochimica della cromatina e delle attività metaboliche, di popolazioni cellulari diverse nei linfociti periferici; a) allo studio, con l'impiego di precursori marcati e tecniche autoradiografiche, dell'attività proliferativa in coltura e delle varie fasi del ciclo cellulare.

Pubblicazioni:

- A. Farulla - G. Naro: Cariotipo e contenuto in DNA in linee cellulari di mammifero in coltura. Soc. Sassarese Scienze Mediche e Naturali. Sassari, 23.2.1972.
- A. Farulla - G. Naro: Osservazioni citogenetiche in soggetti professionalmente esposti alle radiazioni ionizzanti. 9th Annual Meeting European Society Radiation Biology. Roma, 26-28 settembre 1972.
- A. Farulla - V. Monesi - G. Naro: Effetti delle radiazioni ionizzanti sulla sintesi del DNA. Ricerche su linfociti circolanti in coltura. *ibid.*
- A. Farulla - V. Monesi - G. Naro: Studies on the carioϑram of lymphocytes from radiation workers and on some cellular activities of lymphocytes in culture (relaz. inviata al XI Congr. Mondiale dell'IRPA).

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici: Prof. A. Farulla, Prof. C. Biazini, Prof. V. Monesi, Prof. G. Naro.

Titolo del progetto: Effetti della prolungata esposizione a bassi livelli di radiazioni ionizzanti; ricerche morfologiche, citochimiche e citogenetiche sui linfociti circolanti di soggetti professionalmente esposti al rischio delle radiazioni ionizzanti in un impianto nucleare di potenza dell'ENEL.

Dalle 57 colture allestite nel corso del 1972 con sangue periferico prelevato da lavoratori esposti si è avuto lo sviluppo di 3.876 piastre metafasiche, esaminate dal punto di vista quantitativo e qualitativo. Di queste, 3.688 (95%) sono euploidi e 125 (5%) aneuploidi; l'aneuploidia è caratterizzata da una netta prevalenza di cellule ipomodali (171) rispetto alle ipermodali (24). Nessuna delle cellule esaminate mostra aberrazioni cromosomiali stabili o instabili. Anomalie cromatidiche (gaps e rotture) si riscontrano in 232 cellule ossia nel 6% di tutte le metafasi esaminate.

Dalle 13 colture dei soggetti controllo, studiati nel corso del 1972, si è avuto sviluppo di 793 piastre metafasiche; l'incidenza di aneuploidia, anche in questi casi con netta prevalenza della ipodiploidia, è del 5,6% (40 cellule ipomodali e 5 ipermodali), e quella delle cellule con aberrazioni cromatidiche del 3,8% (30 cellule). Anche in questi campioni nessuna cellula presenta aberrazioni di tipo cromosomiale.

Le dosi assorbite dai lavoratori nel corso dell'anno variano da un minimo di 750 mrem ad un massimo di 4.100 mrem, con una distribuzione più concentrata nel periodo maggio-luglio in coincidenza con lavori di refueling e manutenzione. I soggetti in esame hanno accumulato, dall'inizio dell'attività nucleare al momento dell'esame citogenetico, dosi variabili da un minimo di 5 rems ad un massimo di 23 rems.

I dati sopraesposti consentono alcune considerazioni: da segnalare in primo luogo il mancato riscontro di aberrazioni cromosomiali dopo 8 anni di lavoro nucleare comportante una esposizione radiologica a livelli certamente bassi ma comunque molto più elevati di quella cui è esposta la restante popolazione. Permane inoltre nei soggetti esposti un seppur lieve aumento dell'incidenza di aberrazioni cromatidiche rispetto ai soggetti del gruppo controllo. Tale maggiore incidenza non ha tuttavia un andamento uniforme: si osservano infatti differenze tra soggetti che hanno realizzato livelli di esposizione pressochè uguali, e nello stesso soggetto la percentuale di cellule con aberrazioni cromatidiche può variare nel tempo senza un preciso rapporto con il progressivo accumulo di dosi di radiazioni.

Le indagini citochimiche e morfologiche sono state orientate, in questa fase, alla caratterizzazione del contenuto in RNA citoplasmatico e delle modificazioni nucleolari nel corso della trasformazione blastica. All'inizio della coltura sono presenti piccoli e medi linfociti, con i tipici aspetti strutturali, e leucociti polimorfonucleati; nel corso della coltura, mentre questi ultimi subiscono processi degenerativi e diminuiscono di numero fin quasi a scomparire, i linfociti si trasformano progressivamente in cellule di grandi dimensioni di tipo bla

stico; le modificazioni più importanti riguardano la cromatina che diventa meno compatta, i nucleoli che aumentano di grandezza ed il citoplasma che mostra una più intensa basofilia in rapporto ad un cospicuo aumento del contenuto in RNA presente soprattutto come RNA ribosomale; la basofilia scompare dopo trattamento con ribonucleasi.

Le modificazioni descritte sono già evidenti dopo 8 ore dall'aggiunta di fitoem-agglutina alla coltura; nei tempi successivi aumentano sia la percentuale di linfociti in trasformazione che l'intensità dei processi di attivazione. Nelle colture di 48 ore le grandi cellule blastiche intensamente basofili sono cellule predominanti, i piccoli linfociti e i granulociti sopravvissuti sino molto rari, compaiono alcuni macrofagi.

Si è potuto constatare che soltanto una parte dei linfociti circa il 40%) subisce il processo di trasformazione; tale percentuale sale al 60-80% della popolazione mononucleata a 72 ore di coltura per due fattori; a) la moltiplicazione dei linfociti trasformati che inizia a 36-42 ore di coltura; b) la scomparsa per lisi dei linfociti non trasformati.

Non è possibile, allo stato attuale delle ricerche, correlare la capacità trasformante ad un parametro morfologico differenziale nella popolazione linfocitaria iniziale nè definire se la frazione di linfociti che si trasforma rifletta una inomogeneità della popolazione iniziale nei riguardi della potenzialità evolutiva o sia da attribuire ad una selezione casuale in una popolazione omogenea.

L'esistenza di popolazioni linfocitarie diverse è tutta la suggerita, oltre che da ricerche immunologiche, dalle nostre osservazioni sulla variabilità della durata del periodo G₁ che precede l'inizio della sintesi del DNA ed anche dalla dimostrazione che soltanto una parte della popolazione linfocitaria subisce la trasformazione blastica e presenta un aumento della sintesi del RNA.

Contracting party of the Commission :
EUROPEAN LATE EFFECT 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 concentrated his efforts during the year 1972 on the standardization of the experimental conditions in the participating institutions, on the coordination of the planning and performance of on going research projects in the area of radiation late effects, as well as in the unification of specific cooperative projects on carcinogenesis and on dysplasia and dystrophia lesions.

Standardization Committee

- 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-6. In the field of carcinogenesis
- B-7. In the field of the non-neoplastic changes
- N.B. List of coordinated research programmes.

Results of project N° 1.

Head of the project : J.J. BROERSE

Title of the project : STANDARDIZATION OF RADIATION
DOSIMETRY

The first intercomparison project on X-ray dosimetry has revealed a number of discrepancies with respect to the absolute dosimetry and the exposure conditions employed at the various institutes (Puite et al., 1972). These results which are summarized in figure 1, indicated that the dosimetry procedures employed at a number of institutes had to be improved. During a meeting of the EULEP dosimetry group, held at Rijswijk on June 15 and 16, 1972, the X-ray exposure arrangements of the institutes participating within EULEP, have been discussed. After inclusion of a number of modifications the protocol for EULEP X-ray dosimetry and a code of practice for irradiations of small animals were approved. The protocol is available from the chairman upon request; the code of practice is attached as an appendix to this report. A second X-ray dosimetry intercomparison project will be initiated to check the improvements made on the dosimetry at the various institutes. The project will commence in February 1973 and will be confined to the measurement of the dose distribution over a mouse phantom, irradiated in the actual mouse cages employed at the participating institutes.

Members of the dosimetry committee have made site-visits to two laboratories to check the X-ray dosimetry and to perform dosimetry studies for a project of the committee on non-neoplastic changes concerning vascular changes induced by local irradiations of the rat brain.

With regard to the effects of internal emitters, two working groups have been established, which will examine the standardization of dosimetry of intracellular tritium and bone seekers (radium and strontium). Drs. Lambert (London) and Müller (Neuherberg) act as coordinators for the projects on tritium and bone-seekers, respectively. Within the framework of the EBONY project (European Bone-seeker Dosimetry) the procedures employed at the various institutes are explored and a standardization of the following factors will probably be achieved :

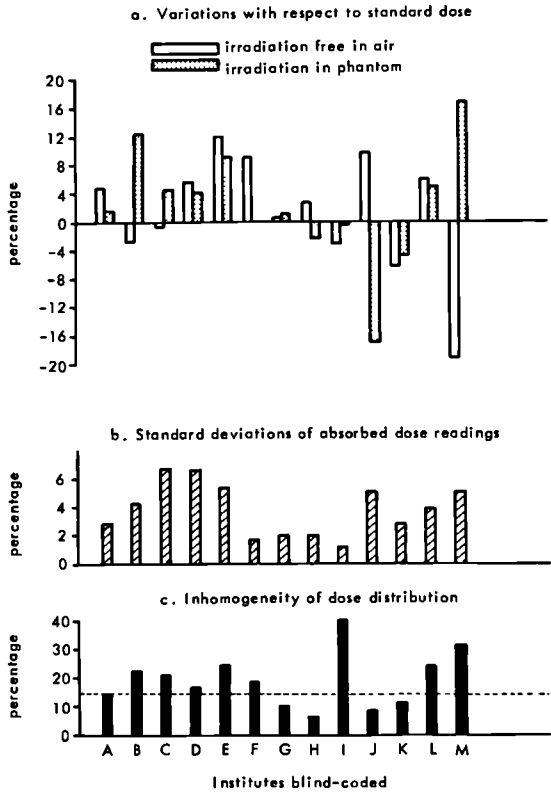
- 1) physical and chemical properties of the radioactive nuclide ;
- 2) mode of application and measuring methods of metabolism ;
- 3) methods employed for the determination of the energy deposited in the bone.

Appendix

CODE OF PRACTICE FOR EULEP X-RAY DOSIMETRY FOR IRRADIATIONS OF SMALL ANIMALS

1. A description of the exposure conditions for X-irradiations has to comprise the following parameters :
 - the quality of the radiation : high voltage, the current of the X-ray machine and the HVL (half value layer).
 - the absorbed dose at the volume of interest or the exposure at that position and the way the absorbed dose was derived.
 - the temporal distribution of absorbed dose (dose rate, fractionation, timing of successive exposures, etc.).
 - the geometrical conditions of irradiation (distance from focus to centre of animal, field size, scattering materials, etc.).
 - the ratio between maximum and minimum absorbed dose in the volume of interest.
2. It is recommended that in the majority of radiobiological experiments class A irradiation should be applied (ICRU report 10e), i.e. a ratio of less than 1.15 between maximum and minimum absorbed dose in the volume of interest. To comply with this condition, the following procedures are suggested:
 - exposure variations over the irradiation field used (in the absence of biological objects) should not exceed + 3% of the average exposure. In some cases rotation of the objects during exposure can give an improvement of the homogeneity of the dose distribution.
 - maximal source-to-object distances should be used in order to diminish the effect of the inverse square law.
 - a sufficient filtration of the radiation should be used (to diminish the effect of the absorption) e.g. an HVL of at least 1.5 mmCu.
 - X irradiations can be carried out in two alternative ways, either on a time-basis employing a mean exposure rate of the X-ray machine, and checked using a monitor ionization chamber or using a monitor ionization chamber checked on a time-basis. The monitor chamber should be placed in the beam line in a fixed position with respect to the position of the irradiated object. The ionization chambers should be checked against a standard ionization chamber at regular intervals of at least once a year.
 - maximum scatter conditions, using unit density material of approximate tissue equivalence (e.g. pressed wool) should be employed.

3. The ICRU recommendations have to be consulted for radiation quantities and units, for values of physical parameters (e.g. rad/R conversion factors of 0.95 and 0.96 for the dose in soft tissue for X-rays and gamma rays, respectively), and in addition for irradiation conditions (e.g. HVL measurements).



Results of first EULEP intercomparison of absorbed dose and dose distribution for X-irradiations using mailed LiF thermoluminescent dosimeters.

Reference

Puite, K.J., Creholder, D.L.J.M., Hogeweg, B and Broerse, J.J.,
 Intercomparison of absorbed dose and dose distribution for
 X-irradiations using mailed LiF thermoluminescent dosimeters.
 Phys.Med.Biol. 17, 390, (1972).

Results of project N° 2.

Head of the project : H.P. SCHNAPPAUF

Title of the project : COMMITTEE ON LABORATORY ANIMALS
STANDARDIZATION (CLAS).

Aims : Recommendations for care and management of laboratory animals (L.A.) in accordance with existing national and international (ICLA) guidelines, with particular respect to irradiation- and long term experiments relevant to the joint studies of EULEP.

Independent test experiments are to be performed in order :

- a) to ascertain the consistency of test results in the particular laboratories ,
- b) to make results of different laboratories comparable.

Situation : Construction of barrier defended, well air conditioned L.A. quarters in the different laboratories will be the major pace maker particularly for success of animals experimentation in the EULEP projects.

From this aspect, the situation is rather discouraging, since for financial reasons, plans to construct adequate L.A. facilities can not be inaugurated in many institutes or have been postponed in others. It is the unanimous opinion of the CLAS that the council of EULEP via EURATOM or any other way should exert some influence on the different national and local authorities responsible for financial support of the respective participating laboratories to make these consider construction of adequate L.A. facilities in the near future.

This is in accordance with trends in two different fields influencing animal experimentation :

- 1) Research foundations and other authorities funding research projects are adopting the policy of supporting only those laboratories which can proof management of L.A. facilities in accordance with national standards.
- 2) A European Animal Protection Act, being discussed already in Strasbourg will eventually allow experimentation with L.A. only if the a.m. standards are met.

A questionnaire action revealed considerable variation in L.A. management of the different laboratories, particularly with respect to air conditioning (relative humidity), hygienic precautions (sterilization of drinking water, food, bedding, cages), effective barriers available and

microbiological control (almost none!). Clearly this situation is not apt to make results of different laboratories comparable; in some cases consistency of results in the same laboratory can hardly be expected.

Particular activities of CLAS

Aside from technical problems of evidencing chronic or latent infection in L.A. colonies, several microbial species not banned by SPF-Lists^{**} interfere particularly with the type of experiments projected by EULEP.

Hardly controllable factors (f.i. changes in food composition, pesticide residues in bedding) may also contribute to unexpected responses of L.A. It was decided therefore that two independent multiparameter tests should be performed, which are likely to pick up factors deleterious to the joint studies :

- a) LD 50/30 repeatedly (tentatively 4 times in the first year). Rather than comparing given LD 50/30 figures (as has been done so far) joint evaluation of basic data (f.i. histograms of deaths versus time) is expected to reveal causes of inconsistencies.
- b) Life span and body weight development, continuously accumulating low threshold factors.

When consistency of results has been shown, both a and b can contribute to the characterization of the different strains used, which in many cases is so far insufficient.

Practical steps

Ad hoc standardization procedures have been set up as a minimum, below which participation in the joint projects can not be recommended. (München Nov. 3rd, 1971 and Reisenburg Dec.18th, 1972).

Procedures for determining LD 50/30 have been set up (München 1971) rediscussed (Mol Nov.10th, 1972, Reisenburg Dec.18/19th, 1972). The laboratories are urged now to turn in the most recent data until April 3rd, 1973. Results and ensuing new steps will be discussed on May 13/14th, 1973 in Louvain.

Preliminary procedures involved in life span determination have been drafted. A seminar on this subject will be prepared for the May-meeting.

** Liste von Erregern zur Spezifizierung bei SPF-Versuchstieren
Soc. Lab. Animal Science, Publication Nr 2, Zürich 1972.

Category 4. The accreditation and recognition schemes for suppliers of laboratory animals.

MRC Manual series No. 1
Carshalton 1971

Results of project N° 3

Head of the project : W. GÖSSNER

Title of the project : COMMITTEE ON PATHOLOGY STANDARDIZATION

In June 1972 the second meeting of the committee on pathology standardization has taken place in Munich.

The main topic of this meeting was a slide seminar on liver tumors. 19 slides of liver tumors have been sent to all participating laboratories and have been discussed during the meeting.

A preliminary classification of liver tumors was proposed and by most of the participants accepted :

1. Mesenchymal tumors and tumor like lesions
 - 1.1. Hemangioma like lesions (probably reactive), sinusoidal - capillary - cavernous pattern, with / without proliferation of mesenchymal (endothelial) cells
 - 1.2. Hemangioma, cavernous
 - 1.3. Malignant hemangi endothelioma
 - 1.4. Reticulo-endothelial sarcoma (Kupffer cell sarcoma)

2. Epithelial tumors and tumor like lesions
 - 2.1. Nodular hyperplasia (hyperplastic nodule) *
 - 2.2. Benign hepatoma (hepatoma) *
 - 2.3. Malignant hepatoma,
 - 2.3.1. well differentiated type (metastasizing hepatoma, well differentiated hepatocarcinoma),
 - 2.3.2. anaplastic type (undifferentiated hepatocarcinoma)

* differentiation purely on morphology is often impossible.

In addition there was a discussion of different problems concerning the slide seminar on bone tumors, which has been held in October 1971 at Munich.

The following classification of bone tumors in experimental animals has been accepted :

1. Osteosarcoma
 - 1.1. Osteoblastic type
 - 1.2. Chondroblastic type
 - 1.3. Fibroblastic type
 - 1.4. Vascular type
 - 1.5. Osteoclastic type (Giant cell type)
 - 1.6. Reticulo-histiocytic type
 - 1.7. Mixed type
 - 1.8. Pleomorphic type
2. Benign osteogenic tumors
 - 2.1. Ossifying fibroma
 - 2.2. Osteoma

The histologic typing of the odontogenic tumors is in accordance with the WHO International histological classification of tumors No. 5.

A picture atlas on bone tumors in mice and rats has been prepared and is now ready to go to press.

In addition to the slide seminar the consultation centre has started to work.

Results of project N° 4

Head of the project : A. KEYEUX

Title of the project : COMMITTEE FOR CLINICAL PATHOLOGY
STANDARDIZATION

The function of the Committee for Clinical Pathology Standardization, as was previously underlined, consists of the promotion of new investigation techniques or adaptation of existing techniques to a well-defined project.

Thus the committee took particular interest during 1972 in the development of two EULEP projects to which EULEP has now given financial support, namely :

- 1) The radiation effect on the Central Nervous System as a model for late vascular changes.
- 2) Production and testing of anti-mouse and anti-rat lymphocyte serum.

Concerning the first one, which will be started in January 1973, the participants agreed upon the use of 2.2.2. tribromoethanol, as an anesthetic drug offering a high degree of safety, in the preparation of the rat for brain irradiation. Another point of interest is the standardization of the various experimental procedures. Three of them are relevant to physiology and biochemistry, and therefore to Clinical Pathology namely :

- 1) The blood dilution curve of a radiotracer as a means to investigate the vascular permeability, the blood flow and the blood volume in the brain.
- 2) The surface fluorometry as a measure of brain metabolism.
- 3) The biochemistry of the brain (biogenic amines, lysosomal enzymes ...).

Each of these experimental procedures will be systematically described and their ability to detect small physiological and biochemical disturbances will be especially emphasized.

In this way, technical assays and pilot studies were performed during 1972.

During 1973, a model for the pharmacological evaluation of anti-lymphocyte serum (ALS) produced by the TNO Radiobiological Institute in Rijswijk, will be proposed to the members of the committee for carcinogenesis. In a first step, acute, subacute and chronic toxicity as well as the effectiveness of "EULEP made ALS" in immunological suppression will be tested on the different strains of animals available for carcinogenesis assays.

Results of project N° 6

Head of the project : J.F. DUPLAN

Title of the project : COMMITTEE ON CARCINOGENESIS

The year 1972 must be considered as transitional. The various laboratories members of EULEP have agreed on a common programme of research which will be started in 1973. It was, therefore, necessary for each Institution to develop its own programme in order to reach a point at which it could be fitted within the general project of EULEP.

For this purpose research has been directed along three main lines :

- 1) Studies on the relationship between the numbers of cells at risk and the incidence of tumors of the hemopoietic tissues.
- 2) Induction of tumors by radioactive nuclides.
- 3) Respective role of viruses and radiation in the development of leukemia.

I. Number of cells at risk and tumors of the hemopoietic tissues

This field is covered by St Bartholomews Hospital (London) and Casaccia (Rome). Both projects are complementary and have established cooperation.

In one case normal non-irradiated mice are injected with a fixed number of previously irradiated bone marrow cells. The dose of radiation received by the bone marrow varies from 100 to 600 R and the number of cells injected is adjusted in order to correspond in all cases to 50.000 living CFU. Thus the number of "cells at risk" is stable while the dose of radiation received by the population is variable.

In the second instance mice irradiated with a sublethal dose of X-rays are injected with various amount of normal isogeneic bone marrow cells corresponding to 50 to 5.000 CFU. Interesting preliminary results have thus so far been obtained with respect to the incidence of reticulum cell neoplasms. The Department of Pathology (Liège) has developed a different system to estimate the type of cells (X cells) which accumulate in thymic cortex of irradiated mice. There seems to be a correlation between the presence of X cells and the probability of leukemic transformation.

2. Induction of tumors by radioactive nuclides

The laboratory of Radiobiology (University of Louvain) is mainly interested in the development of liver tumors while the Research Institute of National Defence (Sweden) works on bone seekers.

The cooperative project is intended to study the role of immunity in the onset and the development of the malignant clones. For this purpose the participating laboratories had to establish the dose-effect relationship (Dosimetry Standardization Committee) and the chronology of the various stages of development of the malignancies. This has been started in 1972 and is well under way. It is worth noticing that the Res. Inst. of Natl. Defence has demonstrated that the tumors induced by 90-Sr contain a new antigenic component which seems to be different for each tumor. Further development of this work implies the use of ALS (Committee of Clinical pathology standardization).

3. Role of viruses and radiation in the development of leukemia

There are two main projects in this field. The Department of Radiobiology (Mol) and the Research Unit of INSERM (Bordeaux) are both working on a potent strain of RadLV called Passage D. In 1972 the techniques have been standardized in such a way that transmission, isolation and purification of the virus have been obtained under similar conditions in both laboratories.

Simultaneously, immunological work has developed in Mol and preliminary studies show the possibility to immunize mice against passage D.

In Bordeaux the main purpose of the studies has been to obtain the in vitro multiplication of the virus and to find a technique for its titration. For this last purpose the use of XC cells and BALB/c mouse fibroblasts has been finally retained and is presently being routinely investigated.

A different model is used in the Department Clinical Physiology (Ulm) where studies are in progress to determine the influence of radiation on the development of leukemia initiated by Moloney leukemia virus.

Results of project N° 7

Head of the project : G. GERBER

Title of the project : COMMITTEE ON DYSPLASIA AND DYSTROPHIA
QUANTITATIVE AND QUALITATIVE CELL CHANGES

The project on irradiation of the central nervous system as a model for late vascular changes was designed to elucidate which role does vascular changes play in the development of late effects in brain, which is the pathogenesis of these changes and whether and how these changes can be prevented : Pilot studies were commenced in 1972 on rats exposed locally to the brain ; the vascular architecture studied in Oxford using a total mount embedding does not show extensive changes after this treatment for a period of several months but other time intervals and doses will now be utilized for detailed studies. Measurements of NADH fluorescence under different conditions of O_2 breathing were developed at Rijswijk. Preliminary results indicate an increased response in irradiated brain.

The determination of vascular permeability and blood flow was set up at Louvain and experiments on irradiated brain have been started. The response of endothelial cells in the brain to conditions of decreased O_2 tension was followed in Ulm using thymidine 3H to determine the cell kinetics. No increased labelling was as yet found after 2-4 days hypoxic atmosphere. More extensive experiments are in progress. Methods to determine biochemical parameters in the brain were set up at Mol. An increase in DNA, sialic acid and cathepsin was observed in locally irradiated rat brain. Simultaneously, uptake of α -aminoisobutyrate was found diminished in muscle and heart, perhaps as a result of changes in the regulatory reactions of the brain.

N.B. COORDINATED RESEARCH PROGRAMMES

List of the projects in progress in the different laboratories, coordinated by EULEP but not supported financially.

UNIVERSITÄT ULM - MEDIZINISCH-NATURWISSENSCHAFTLICHE HOCHSCHULE -
ZENTRUM für KLINISCHE FORSCHUNG

1. Pathogenesis of the late haematopoietic effects after single, repeated and continuous whole body radiation exposure with special emphasis on the radiation effects on the bone marrow stroma. (Stem cell and oocyte effects after x-irradiation and exposure to tritium; marrow regenerating ability after mechanical marrow disruption; studies in man after thorotrast incorporation).
2. Turnover of the elements of the vascular system and its relation to the pathogenesis of radiation late effects (establishment of methods to study quantitatively the turnover of blood vessels; cell survival studies of blood vessels).
3. Studies on the pathophysiological mechanisms of leukemia development in mice using the Rauscher disease as one model for investigating radiation leukemogenesis as a consequence of external and internal ionizing radiation.

DEPARTEMENT DE RADIOBIOLOGIE - C.E.N. - MOL

- Late effects of radiation on mouse lung (changes in the ultrastructure and capilar permeability).
- Characterization of the preleukemic stade in the mouse.

RADIOBIOLOGICAL INSTITUTE TNO and the INSTITUTE FOR EXPERIMENTAL
GERONTOLOGY TNO

Late effects of radiation on mouse lung
Late effects of radiation on rat skin
Late effects of radiation on rat spinal cord
Late effects of radiation on bloodvessels of the rat
Late effects of local radiation on blood flow in the mouse tail
Late effects of radioactive iodine on rat thyroid.
Mammary tumor induction in rats under influence of radiation
and other agents
Leukemia induction in rats and mice under influence of radiation
and other agents.

LABORATOIRE DE RADIOBIOLOGIE - INSTITUT DU CANCER U.C.L. - LOUVAIN

Late radiation changes in the cardiopulmonary function.
Late circulatory and functional changes following irradiation of the
liver.
Tumor inducing properties of ionizing radiations and chemical mutagens
in the liver.
Late radiation effects on the lymphoid system.
Late circulatory changes in the irradiated skin.
Sodium turnover in irradiation hypertension.

DEPARTMENT OF RADIOBIOLOGY - INSTITUTE OF NUCLEAR RESEARCH - WARSAWA

Delayed post-irradiation damages of collagen.
Late impairment of proliferation of mouse lymphoblasts irradiated with
X-rays and ultraviolet.

RESEARCH INSTITUTE OF THE SWEDISCH NATIONAL DEFENCE

Autoradiographic, metabolic and pathologic studies of radio-barium.
Dose related pathology of ^{241}Am .

Different projects on the late effects of radio-strontium)
(influence of various hormones, age, cold, ...).

Possible synergistic effects of external X-irradiation and ^{90}Sr -
injections and the induction type, latency time and origin of
leukemia.

The effects of ^{90}Sr on the oocytes of fetal mice.

Late effects of 14 MeV neutrons.

Pathology of radioruthenium.

Possible effects of high radioiodine doses on thyroideal C-cells and
its influence of ^{90}Sr retention.

DEPARTMENT OF RADIOBIOLOGY -

MEDICAL COLLEGE OF ST. BARTHOLOMEW'S HOSPITAL - LONDON

Factors affecting lifespan in laboratory mice.

Mechanisms of leukaemogenesis in outbred SAS/4 mice, inbred CBA; and
viral transmitted leukaemia in RFM mice.

The natural history of myelogenous leukaemia in RFM mice, to study interaction
of leukaemogenic development with normal haemopoietic stem cells of bone
marrow or splenic origin.

Mechanisms of lung tumour induction in SAS/4 (outbred), and C3H (inbred) mice.

Factors affecting the response and metastatic spread of spontaneous mammary
tumours in C3H mice.

Late effects of wholebody irradiation to 14MeV X-rays, Co^{60} γ -rays, and
14MeV neutrons, on CFU efficiency, in 18-20 month old mice.

Effect of tritiated thymidine incorporated during foetal and postnatal life
in rats (from Ulm), on lung cell populations.

GRUPPE BIOLOGIE ISPRA
KOMMISSION DER EUROPÄISCHEN GEMEINSCHAFTEN

BIOLOGY GROUP ISPRA
COMMISSION OF THE EUROPEAN COMMUNITIES

GROUPE DE BIOLOGIE ISPRA
COMMISSION DES COMMUNAUTÉS EUROPEENNES

BIOLOGY DIVISION ISPRA/ITALY
Directorate for Biology of the
Commission of the European Communities

K. Gerbaulet

DIRECT PARTICIPATION OF THE COMMISSION IN
ITS PROGRAMME BIOLOGY-HEALTH PROTECTION

GENERAL DESCRIPTION

The research work carried out in 1972 was pursued according to the established programme and closely linked to that of various EURATOM Association or Group Contracts.

A partial extension of the environmental studies to "non nuclear" aspects became necessary because of the additional influences of conventional pollutants when evaluating risks arising from effluents of nuclear installations, and the request to participate in the Joint Research Centre's (J.R.C.) own Environmental Protection Programme.

PROJECT 1. Environmental contamination by radioactive and conventional pollutants. The studies were mainly concerned with the transfer and effects of heavy metals in/on terrestrial and aquatic ecosystems. Chromium was chosen as pollutant for a pilot study because of its release in both forms (radioactive and non-radioactive) from nuclear plants. This work was carried out in collaboration with the Association EURATOM-ITAL, in the Netherlands. Further work, executed on request of the Association EURATOM-CEA, France, considered specific aspects of the transfer of radioactive zinc, mercury, cesium and iodine. Studies on the effects of various conventional heavy metal pollutants were also performed in collaboration with the J.R.C.

PROJECT 2. DNA damage by radiation and mutagenic chemicals. Studies of the effects of radiation and chemicals on nucleic acids were continued in the framework of the "Liaison Group on Genetic Effects of Radiation". Isolated mammalian systems were used in order to elucidate the mechanisms primarily involved in the enzymatic expression and repair of DNA damage. Part of the work was a joint effort with the Chemistry Division of the J.R.C., the Laboratorio di Genetica Biochimica ed Evoluzionistica of the CNR in Pavia/Italy, and the Medisch Biologisch Laboratorium of the TNO in Rijswijk/Netherlands.

PROJECT 3. Radiation Biophysics and Microdosimetry. A participation in the coordinated research programme of the "Contractors Group Dosimetry" was continued. Experimental and theoretical studies on the radiation structures of alpha particles, protons, deuterons and electrons in biological material, and in model substances, were performed in close collaboration with Member Institutes. On the basis of the structures obtained, ionization patterns and energy deposition patterns of fast neutrons and X-rays in critical biological volumes were evaluated.

PROJECT 4. Radiation sensitivity of insects. The physiological effects of gamma-irradiation on Lepidoptera at different stages of life-cycle were studied. Adequate rearing procedures under laboratory conditions were elaborated for this species. The work was carried out in close collaboration with the contractors of the EURATOM Radioentomology Programme and particularly within a contract with the University of Padova/Italy.

It should be emphasized here that the research was considerably assisted by a close collaboration with the multidisciplinary Divisions of the Ispra Establishment of the J.R.C. and by the availability of Fellowships and student grants.

RESULTS OF PROJECT 1

Head of Project and Scientific Collaborators : K. Gerbaulet
M.F. Baudouin, A. Berg, E. Levi, C. Myttenaere, O. Ravera,
P. Reiniger, P. Scoppa and G. Ferrarese*, W. Penning*,
J.Y. Standaert*

Project Title : ENVIRONMENTAL CONTAMINATION BY RADIOACTIVE AND CONVENTIONAL POLLUTANTS

A. Transfer of radioisotopes in terrestrial ecosystems.

- Direct contamination studies of bean and lettuce leaves using single drops or spraying under controlled environmental conditions indicated that ^{51}Cr whether applied in the cationic trivalent or the hexavalent anionic form penetrates the leaves and accumulates at the treated areas without any significant translocation. The fractions retained or which subsequently could be washed out or exchanged varied with the chemical form applied.

- Retention, adsorption, absorption and movement of ^{137}Cs and ^{65}Zn were measured in bean leaves following foliar application under controlled conditions. In the period 1-168hrs after treatment, Cs alone was taken up and translocated to all parts of plants; losses from roots were also registered, whereas Zn accumulated only at the treated areas. Mixtures of the two elements showed an antagonistic reaction of Zn on the movement of Cs in beans.

- A large scale experiment compared the retention of ^{137}Cs and ^{65}Zn when sprayed separately on mixed stands of rye grass and clover grown and kept during the experimental period either in a growth controlled room, in a greenhouse or in field conditions. The influence of repetitive applications was also determined. Results show a large similarity in retention capacity of plants grown in the various environmental conditions, and a relative similarity between the percentages of Cs and Zn retained by either species when calculated per unit fresh weight.

- The metabolism of ^{51}Cr following root uptake was determined in tomatoe and rice plants as a function of both the chemical form applied (tri- or hexavalent Cr, or its EDTA-chelate) and the concentration of stable
* Post-graduate students.

Cr. Absorption and total plant content of ^{51}Cr was much higher for the cationic and anionic forms than the chelated one and increased correspondingly with an increase in the stable chromium of the nutrient solution. The major fraction of the radioactivity was localised in the root systems (in tomatoe, in the fine secondary roots) and only a negligible fraction reached the fruits. About 50% of the ^{51}Cr EDTA-chelate was absorbed and translocated to the aerial parts. An examination of the sub-cellular distribution showed a preferential binding to membranes.

- The transfer of ^{131}I from irrigation water to grass was studied in a model simulated grassland. When $1\ \mu\text{Ci}/\text{ml}$ of ^{131}I was present in the irrigation water, a water to grass transfer coefficient of about $0.08\ \mu\text{Ci}/\text{g}$ was found. The relative distribution of the radioactivity within the plants was roughly: roots = 100, stem-bases = 40 and shoots = 2. The vertical distribution of ^{131}I in the soil profile decreased exponentially. Similar experiments using ^{51}Cr and ^{65}Zn in the irrigation water resulted in a similar repartition of both isotopes in the various fractions studied: 60-70% of the radioactivity was fixed by the soil, plant-mat and grass. The concentration factors grass/water were about 10 for Zn and 3 for Cr. Successive plant harvests showed that only in the case of ^{51}Cr an increase in total radioactivity of the grass could be observed even after irrigation with non radioactive water.

- The behaviour of ^{51}Cr was examined in a calcareous river-clay soil as a function of the chemical form applied. More than 99% of the cationic trivalent form was "fixed" by the soil, about 40% of which could be extracted by 1% citric acid, but less than 1% by 1N CaCl_2 or ammoniumacetate at pH 7. When applied in the anionic hexavalent form the "fixation" of ^{51}Cr followed a saturation curve with a fraction, between 30 and 40%, remaining in solution at the end of the experimental period of 130 hours.

- The influence of soil moisture tension on the uptake of ^{137}Cs was studied in bean plants grown on a podzol: the concentration factor established at a tension of 0-2 bar was decreased by a factor of 2 when the soil moisture tension was increased to 6 bar.

The method of soil contamination was also found to influence the uptake of ^{137}Cs by bean plants. In a terra fusca with a high Cs-fixing capacity no effect was observed; whereas in a loess and podzol the concentra-

tion factors established after fresh contamination were decreased by a factor of 2-4 in the second crop.

- An analysis of the chemical composition of soil solutions of the six soils used in the Eurosoil Project was terminated; the vertical concentration profiles were established for calcium, magnesium, sodium and potassium.

B. Transfer of radioisotopes in aquatic ecosystems.

- Transfer studies of ^{51}Cr , ^{65}Zn and ^{203}Hg from water to planktonic algae (*Selenastrum capricornutum*) revealed concentrations factors (algae/water) which decreased by a factor of about 10 from Hg to Cr to Zn. When feeding these radioactive algae to adult zooplankton (*Eudiatomus padanus*) the corresponding concentration factors (zooplankton/algae, calculated for unit fresh weight) were observed to decrease by a factor of about 2 in the sequence of Zn to Hg to Cr.

- A comprehensive study was carried out on the transfer of ^{51}Cr -labeled chromic and chromate ions from water to zooplankton. Concentration factors, uptake and release kinetics were established for *Daphnia Hyalina*, *Cyclops strenuus* and *Eudiatomus padanus*. The concentration factors were observed to be always lower than 100, despite non negligible surface adsorption. Retention curves for radioactive chromium consisted of at least two components. The short-life component accounted for the elimination of more than 90% in 24 hours. These results show that the transfer water to zooplankton is not critical for the accumulation of chromium through this aquatic food chain.

- Studies on the accumulation of ^{65}Zn by freshwater fish were pursued. It was found that an addition of chelating agents (EDTA) to food increased the intestinal retention but decreased sharply the retention resulting from direct absorption, because of an inhibition of the Zn exchange between blood and tissues. The ionic form (Zn^{++}) was found to be re-

sponsible for the uptake as proved in artificial water conditions; any exchange of stable Zn is regulated independently from the Zn concentration in water. The high accumulation rate of ^{65}Zn in *Lebistes reticulatus* might be used for the determination of concentration factors at equilibrium.

- The transfer of ^{51}Cr from water to two different species of freshwater fish was studied applying either the cationic trivalent (chromic) or the anionic hexavalent (chromate) form and discriminating between direct and intestinal absorption. The results indicated a rapid and irregular uptake rate with an "equilibrium" (saturation condition) being reached after a few days, and a rapid and nearly complete excretion mainly via feces. The accumulation rate of the trivalent chromium is higher compared to that of the hexavalent one. One may conclude from these experiments that the retention of radiochromium is not the consequence of a physiological uptake of an essential element but of an unspecific and easily reversible binding to organic molecules.

C. Biological effects of heavy metal pollutants.

- Comprehensive studies on the toxicity of various metal salts on three planktonic species: *Daphnia hyalina*, *Cyclops strenuus* and *Eudiaptomus padanus*, were carried out under controlled environmental conditions (temperature, oxygen, light, hardness of water, pH, conductivity etc). The physico-chemical properties of each single metal salt were also considered. The concentrations causing 50% mortality within 48 hours were determined for Hg, Cu, Zn, Cr, Cd, Co, Pb, Ni, Cs, Sr. The sensitivity of *Daphnia* to the elements studied was found to be one or more orders of magnitude higher as compared with copepods. The effects on toxicity observed after increased water temperature did not allow any generalized assumption. Synergistic effects of equitoxic doses of various mixtures of metal salts are under investigation.

- The detoxication mechanism for the elements of the II,B subgroup of the periodic table were studied in experimental animals. Zn, Cd and Hg were found to be accumulated predominantly in the liver and kidney,

where they are associated mainly with the cytoplasmic soluble fraction. Chromatographic properties of soluble fractions obtained from rat and fish organs were compared and a metal binding protein was isolated from their liver and kidney. In fish tissue preparations the toxic elements Cd and Hg are primarily bound to a low molecular weight protein similar to metallothioneins of mammals. Liver and kidney content of this metal binding protein could be increased several fold by repeated administrations of small doses of cadmium salts in both species. After such a treatment the nephrotoxicity of mercury chloride in the rat was significantly reduced. Therefore, the biosynthesis of this metal binding protein may represent a biological defense mechanism. The interferences observed "in vivo" between the metabolism of zinc, cadmium and mercury can be accounted for by an intracellular competition for the binding sites of metallothionein.

- Various hepatic drug metabolizing activities were studied in the liver of lead poisoned rats. The results obtained show severe reductions of cytochrome P-450 level, aminolevulinic acid dehydrase, and mixed function oxidase activities. Intensity and duration of these effects were dose dependent. Therefore, in lead poisoned organisms an enhanced sensitivity to foreign chemicals should be expected. Simultaneous exposure to a large number of organic compounds, either accidentally or for therapeutic purpose, may result in non predictable toxic effects.

RESULTS OF PROJECT 2

Head of Project and Scientific Collaborators : F. Campagnari, L. Clerici,
and S.A.M. Bekkering-Kuylaars*, M. Mathelet*, M. Talpaert*

Project Title : DNA DAMAGE BY RADIATION AND MUTAGENIC CHEMICALS. MAMMALIAN MECHANISMS PRIMARILY INVOLVED IN THE ENZYMATIC EXPRESSION AND REPAIR OF THIS DAMAGE.

- The number average molecular weight, M_n , of non-homogeneous DNA samples subjected to zone ultracentrifugation can be calculated as $M_n = M_{max}/2 - a$. In the derived expression, M_{max} is the molecular weight of the DNA fraction at the peak of the sedimentation profile and a is the exponent of the empirical equation $S = kM^a$, which is used to compute the molecular weight, M , of a nucleic acid from its corrected sedimentation coefficient, S . The validity of these assumptions was experimentally proved by comparing the measurements of M_n with direct estimates of the average chain length in DNA. The latter data were obtained from differential determinations of the total and terminal phosphorus in the nucleic acid by means of neutron activation analysis. The new method was applied to X-irradiated DNA for detecting the M_n of its strand fragments, thus allowing to infer the exact number of the induced mono- and bi-helical breaks.

- Poly-dA paired to oligo-dT₈ or oligo-dT₁₀ were synthesized and served as an "open" template to assay the 3.39S DNA polymerase. Radioactive polydeoxynucleotides linked to cellulose were used as substrates to test the purified enzymes for the residual contamination by nucleases.

- The extraction and the partial purification of the replicative DNA polymerase from the nuclei of calf thymocytes were improved. In the absence of detectable endonucleasic activity, the enzyme uses bihelical DNA as a template and may effect net synthesis of DNA by duplicating repeatedly the initial amount of the primer.

- Studies concerning the isolation and characterization of the DNA ligase from calf thymus were extended. Disc electrophoresis on polyacry-

* Post-graduate students.

lamide gel in the presence of sodium dodecylsulfate revealed that the most purified preparation contained essentially a single type of polypeptide chain with a molecular weight of 50,000.

- The number of phosphoryl groups released as inorganic phosphate from DNA or polydeoxynucleotides X-irradiated in aqueous solutions was 8.5 times higher than the number of those converted into phosphomonoesters by the formation of strand interruptions. Free inorganic phosphate and monoesterified phosphate did not increase linearly with the radiation dose in DNA samples exposed to thermal neutrons.

- The priming activity of DNA for the deoxynucleotidyltransferase was correlated with the actual concentration of DNA molecules instead of with the w/v concentration of the nucleic acid. It appeared that mildly irradiated DNA exerts a powerful competitive inhibition on the functional binding between the cytoplasmic DNA polymerase from calf thymus and the normal DNA templates.

- Poly-dA : poly-dT, poly(³H)dT-celluloses, of which one of the poly-dT moieties had been alternatively exposed to X-rays, were assembled stepwise and subjected to the action of DNA ligase. The complex polymers mimicked DNA's carrying a monohelical scission with the radiation injury specifically located at one of its sides. An analysis of the terminal groups present in the irradiated portion of the dT strand revealed that the ligase was able to seal such fraction of the chain breaks which was not of the normal 5'-PO₄//3'-OH conformation and had a polydeoxynucleotide end damage.

- A number of compounds known to hinder the enzymatic polymerization of nucleotides were tested as inhibitors of the catalysis mediated by the RNA polymerase A recently purified from calf thymus nuclei. Rifamycin, rifampicin and rifamycin SV did not cause inactivation of the mammalian ribonucleotidyltransferase; several semisynthetic products, chemically derived from rifamycin SV were found to inhibit the reaction catalyzed by the RNA polymerase A and seemed to interact directly with the enzyme molecules.

RESULTS OF PROJECT 3

Head of Project and Scientific Collaborators : J. Booz

M. Coppola and U. Borst,* R. Eickel,* H. Menzel,* A. Waker*

Project Title : RADIATION STRUCTURE OF IONIZING RADIATIONS IN TISSUE
AND ITS RELATION TO THE BIOLOGICAL EFFECTIVENESS

A. Radiation Structure in Biological Material and in Model Substances

- A scatter chamber has been built for the experimental determination of the radiation structure (ionization) of fast ions and their delta-rays in tissue equivalent gas. The ions (narrow beam of about 1 mm diameter) are flying through a long cylindrical tube of 4 mm diameter which is in the center of the scatter chamber. The tube is connected with the rest of the chamber by small gaps of variable length through which some delta-electrons may pass. The charge produced by these delta electrons outside the central tube is measured on 4 wire electrodes that may work as proportional counters or as ionization chambers. In case of charge multiplication (proportional counter) the gaps are made so small that only one delta electron is passing within the measuring time with high probability. By varying the gas pressure the spectral and local distribution of delta electrons is measured. The chamber is ready to be mounted in front of the accelerator. Preliminary measurements have been done with natural alpha particles of 5 MeV.

- The energy dependence of the differential w-value of alpha particles, w_{α} , in tissue equivalent gas has been measured for monoenergetic alpha particles of 0.27 MeV to 5.3 MeV. Above 1.5 MeV w_{α} is very close to W_w , the constant W-value of electrons. The small difference between the two values can be neglected without making serious errors. Below 1.0 MeV the value of w_{α} is increasing steadily and is at 600 keV about 10% higher than W_w .

* Post-graduate students.

- Another scatter chamber has been constructed for experiments with low energy electrons ($E = 5 \text{ keV}$). It is the aim of this work to measure the local distribution of ionization and the event number distribution of scattered electrons due to multiscattering of a monoenergetic pencil beam of primary electrons in tissue equivalent gas. In addition the energy range relationship, the transmission, and the W-values will be measured. The apparatus can be broken down into three sections, the electron source, an intermediate chamber, and the scatter chamber, all three being differentially pumped. The dimensions of the apparatus have been chosen so that a pressure range of 10^{-2} Torr to 10 Torr is sufficient to enable all the measurements to be carried out on electron beams of 100 eV to 5 keV.

B. Evaluation of the Energy Deposition of Different Types of Radiations to Small Biological Volumes and its Relation to the Corresponding Biological Effectiveness.

- Experimental and theoretical studies on the energy deposition of X- and gamma-rays in cylindrical volumes have been initiated. For the experimental part a wallless counter has been constructed, the theoretical part is a Monte Carlo-study. By comparing the experimental and the theoretical results it is possible to control and adapt the input data set of the calculation, and to extrapolate finally the calculation to volume sizes where experimental determinations are no longer possible.

- A theoretical study on the tissue equivalence of the Shonka-plastic and the Rossi Failla-gas was done by calculating the energy deposition spectra of fast neutrons between 0.6 MeV and 6 MeV in spheres of $1 \mu\text{m}$ diameter for ICRU-tissue and for the mentioned model substances. For fast neutrons below 3 MeV the Shonka-Rossi-material was sufficiently tissue equivalent. Above 3 MeV the model substance was not tissue equivalent when the hypothesis of the dual radiation action was used as criterium.

- The spectral distribution of energy deposition of monoenergetic fast neutrons of 50 keV to 6 MeV to small spheres of 0.1 μ m to 6.5 μ m was calculated by Monte Carlo-methods. The calculation that took into consideration the energy fluctuation of the recoil protons and alpha delivered also results on the energy distribution of the seen recoil ions, the event and dose distribution of LET, the influence of multiple neutron scattering in the surrounding medium, and the neutron spectrum of those primary and scattered neutrons that gave rise to an energy deposition in the sensitive volume. Actually plans are being studied to modify this Monte Carlo-program in order to calculate all above mentioned spectra and parameters at arbitrary positions within a human phantom and for fast neutrons until 20 MeV.

- In order to compare the calculated results with experimental spectra it is necessary to know precisely the spectrum of the experimental neutron beam at the point of interest. For this purpose a He³-counter has been assembled and a new type of proton recoil spectrometer has been developed. Preliminary results show that the width of the measured neutron peak corresponds roughly to the variation given by the experimental conditions, e.g. target thickness, fluctuation of accelerator energy, counter resolution.

RESULTS OF PROJECT 4

Head of Project : R. Cavalloro

Project Title : RADIATION SENSITIVITY OF INSECTS

The sensitivity to gamma radiations was studied in *Sesamia nonagrioides* (Lefebvre) at different stages of life-cycle. Following results were obtained : 36 hours old eggs did not hatch after a radiation dose of 3500 rad; the same effect was achieved with 34000 rad on 120 hours old eggs. The doses necessary to impede adult emergence from 5 day old chrysalis were 300000 rad and increased slightly when irradiating 2 days before the end of metamorphosis. For the adults, females proved to be more sensitive than males; the doses causing mortality in 24 hours were 325000 and 375000 rad respectively. The sterility of vigorous males required doses of 42000 rad when irradiating in the chrysalis stage 2 days before adult emergence, and of 47000 rad when irradiating directly the newly emerged adults. At these doses, the eggs deposited by normal females mated with sterile males did not hatch, moreover ovodeposition was reduced to one-half.

Adequate and effective methods for adult maintenance and larval rearing under laboratory conditions were elaborated.

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ITAL UND UNTERVERTRÄGE

ITAL AND ITS SUBCONTRACTS

ITAL ET SOUS-CONTRATS

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

Nummer van het contract: 094/72/1/BIAN

Hoofd van de researchteams: Dr.Ir. D. de Zeeuw.

Algemeen onderwerp van het contract:

APPLICATIONS OF NUCLEAR METHODS IN BIOLOGY AND AGRICULTURE

- movement of pollutants through soils.
 - uptake, transport, redistribution, accumulation of elements in plants.
 - mutation breeding, incompatibility, mutagenesis.
 - food irradiation.
 - genetic control of insect pests.
 - development of nuclear methods.
-

Algemene omschrijving van de uitgevoerde werkzaamheden:

In the soil and plant part of the programme, mainly the following aspects were studied:

- A simulation model concerning the movement of nitrogen in soils, in relation to fertilization and eutrophication problems, has been further improved.
- Information was obtained on the nitrogen transformations in a water-soil system, on the ecology of rice soils in relation to nitrogen and on the role of nitrogen fixing blue-green algae in the eutrophication of water.
- Data are available on the movement, adsorption and remanent effect in soils of the "non-mobile" insecticide Trichloronate and the "mobile" herbicide Bromacil. Simulation models, considering respectively only convection or convection and diffusion of Bromacil, were worked out.
- The movement of potassium and phosphate ions between, on the one hand, the soil and the soil solution and, on the other hand, the soil solution and the root surface have been simulated, taking into account such parameters as water movement, adsorption, cation exchange, etc. The actual experimental approach mainly considers the uptake of ions from the soil solution, bathing roots and root hairs.
- The study of the kinetics of ion uptake by intact plants is the next step in the movement of the ions through the soil and plant complex. Emphasis was put in 1972 on the relation between hydrogen consumption and anion-uptake
- Further information was obtained on the mechanisms (mainly metabolic) of phosphate transport, distribution and accumulation in intact plants.
- Finally isolated chloroplasts have been used as a model for the ion movement over plant-membranes. Results concerning calcium uptake and release in steady state and non-steady state conditions became available.

In the field of mutation breeding, incompatibility and mutagenesis the following topics were considered:

- The advantages of the combination of the adventitious bud technique and the mutation breeding method of vegetatively propagated crops were again stressed. Several new commercial mutants of ornamentals were brought on the market. It was confirmed that the adventitious buds originate from a single cell, which observation may explain the absence of chimeras.
- A new method, for producing complete plantlets from capitulum-explants

of Compositae and a few other families, will also be of considerable help in the mutation breeding of vegetatively propagated crops.

- The gene of the unstable chlorophyll mutant C₁₁ in tomato (*Lycopersicon esculentum* Mill) has been tentatively located on chromosome 4.
- Self compatible plants, obtained by chronic gamma-irradiation of a self-incompatible dihaploid clone of *Solanum tuberosum* L, have a chimaeric structure, which is probably due to a mutation in the embryo.
- Chronic γ -ray irradiation of tobacco plants (*Nicotiana glauca*, Link and Otto) at the flowering stage appears to be far less effective than acute X-ray treatment for inducing self-compatibility mutations. All mutations induced after X-ray irradiation of pollen mother cells are of a negative nature (genetic losses or showing competition effects leading to self-compatibility). According to the results now available, both, chronic and acute irradiations do not generate new-self-incompatible alleles.
- Research on the marker mutations and identification of the S-bearing chromosome of incompatible plants in *Nicotiana glauca* Link and Otto is in progress.
- This also applies to work on the possible relationship between the S-genotype and the peroxidase isoenzyme pattern of leaves, callus tissue and styles of tobacco and tomato-plants.
- Both, changes in the metabolic activity and induction of a "protective" agent have been considered for explaining the irradiation-dose fractionation effect observed in *Saintpaulia* leaves.
- Using *Lemna minor* L. populations grown on either ¹⁴N or ¹⁵N and either ¹⁰B or ¹¹B solutions and chronically irradiated with thermal neutrons, it was shown that these populations are more sensitive to boron than to N-substitution. Damages due to the N,p (¹⁴N) reaction, however, more severely impair the subsequent growth than damages due to the N, α (¹⁰B) reaction.

In the food-irradiation part of the programme the following results have to be mentioned:

- It is possible to adapt radiation sensitive strains of microorganisms from *Salmonella cameronensis* skin, to irradiated medium, by repeated passage through medium, irradiated with 100 and 200 krad of ⁶⁰Co γ -rays.
- Heat resistance of *Bacillus subtilis* ATCC 6633 increased, radiation resistance and sensitization by pre-irradiation to a subsequent heat treatment, were unaffected by an increase of Mn-content of the sporulation medium. Calcium at 136 ppm. in the medium acted in a similar way. These results are again in agreement with the idea that different mechanisms are responsible for the heat- and irradiation resistance of spores.
- Different types of irradiated media retard or inhibit the growth of *Microbacterium thermosphactum* to various extents; among the factors involved in this process the water availability or "water-activity" of the medium has to be mentioned.
- Practical applications of the food irradiation procedure have been extended to a certain number of different foodstuffs of economic importance.
- In the field of wholesomeness of irradiated food, a total diet feeding test with domestic pigs is in progress.

The programme on radiation genetic control of insect pests progressed in the following way:

- Final study of the conditions which influence the induction of chromosomal rearrangements in the onionfly *Hylemya antiqua* Meigen.
- Working out of simulation models respectively on the theoretic evaluation of different methods for genetic control of the onionfly in the natural

environment and on the possibilities of the release of two independent translocation stocks.

- Research on the repair of the radiation damage after treatment of male and female moths of *Adoxophyes orana* F.R. with substerilizing dosages of X-rays.
- Induction of dominant lethals, recessive lethals and structural chromosome mutations at low doses of X-rays in *Tetranychus urticae* C.L. Koch. A method was developed, by which recessive lethals can be distinguished from structural chromosome mutations. Repair mechanisms after irradiation were also studied.

From the methodology programme, the following aspects are worthwhile mentioning:

- Building and testing of a scanner, which permits water movement and density measurements in undisturbed soil columns.
- Several electronic devices to be used with different analytical apparatus.
- Measurement of density gradients and velocity in flowing granular material.
- Moisture content in plant material, using the absorption of soft β -radiation.
- The method for in-depth localization of β -emitters has been further improved and its application extended to different isotopes, of importance in biology, but having complex decay schemes. The method also permits an estimation of the in-depth distribution of labelled material.
- The former method, as well as macroautoradiography and track-autoradiography have been applied to the problem of calcium translocation in fruits and fruitstems of *Phaseolus vulgaris* L. and *Capsicum annuum* L.
- *Lemna minor* L. bulk populations, respectively grown on ^{10}B and ^{11}B in the presence of pure ^{15}N were submitted to thermal neutron irradiation and given ^{14}C -sodium bicarbonate. Analysis of the material for cellulose, pectic substances, sugars etc. suggests that boron is localized and acts at the level of the cellulose membranes.
- The improved neutron activation analysis method has been submitted to several test runs, using biological material of different origin.

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

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At the end of 1972 Prof.Dr.Ir. P. Buringh retired from the Board and the Management Committee. His successor is Ir. J.B. Ritzema van Ikema (President of the Board of the Agricultural University).

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In the course of 1972, Prof.Dr. F. D'Amato and Prof.Dr. W. Pilnik, respectively vice-chairman and member, retired from the council.

Changes in the Scientific Staff

- Ir. J. Beek, Dr.Ir. R.B. Contant, Dr. R.M. Ecochard and Drs.Mrs. K.J.A. Wijnands-Stäb, have left the Institute, mainly to accept research or teaching duties elsewhere in the world.
- New members of the scientific staff are: Dr.Ir. J. Sinnaeve and Drs. G. Desmet, both from Belgium.

- Temporary members (post-graduate fellows) responsible for particular aspects of the programme: Ir. Miss H.M.G. Groot, Miss M. Matteoli, Ir. C. Petit, Ir. J.P. Rolland and Ir. M. van de Steene.
- Several guest-workers, mainly from developing countries have spent 6 to 12 months at the Institute.

The programme for 1972 has once more been carried out in close cooperation with other scientific institutes wherever possible.

Examples of this scientific collaboration are:

- on heavy elements in the food chain with the Biology Division in Ispra and within the European Society of Nuclear Methods in Agriculture (ESNA);
- on mutation breeding, incompatibility, protein content and disease resistance in higher plants in the Mutation Breeding Contact Group;
- on pollution problems with Institutes in the Netherlands, Germany and Belgium and with the ESNA working group;
- on radiation effects within the European Dosimetry Working Group;
- on standardization of absorbed dose and dose distribution measurements within the European Late Effects Project Group (EULEP);
- on incompatibility in higher plants with several Institutes and Organizations in Italy and the Netherlands;
- on improvement of the "wholesomeness-testing" technique in collaboration with Institutes in Denmark and the Netherlands;
- cooperation to projects concerning the testing of irradiated food, set up by the Organization for Economic Cooperation and Development (OECD) and the International Atomic Energy Agency (IAEA);
- on applications of the food irradiation technique with different Institutes in the Netherlands;
- research on genetic control of insect pests, coordinated, nationally in Section VII of the TNO Working Group "Integrated Control of Insect Pests" and, internationally in the joint European Working Group of the "Organisation Internationale de la lutte Biologique" (OILB) and of ESNA.

Other interesting contacts are those within the German-Dutch cooperation, mainly on food-irradiation, and with groups in the Agricultural Faculty of the Catholic University at Louvain.

Resultaten van het project No. 1

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel.

Titel van het project: Quantitative description of the behaviour of nitrogen in soils.

Beschrijving van de resultaten:

The report 'A simulation model for the quantitative description of the behaviour of nitrogen in soils' will be published by PUDOC, Wageningen, in the series 'Simulation Monographs' as 'Simulation of Nitrogen Behaviour in Soils', edited by J. Beek and M.J. Frissel. The time of M.J. Frissel, available for this project, was entirely spent in adapting and rewriting the initial text.

Publication:(in press)

J. Beek and M.J. Frissel, Simulation of Nitrogen Behaviour in Soils, PUDOC, Wageningen.

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project No. 2.

Hoofd van het team en wetenschappelijke medewerkers:

M.J. Frissel, P. Poelstra, F. van Dorp, L. Stroosnijder, J.G. de Swart.

Titel van het project: Transport of Soil Moisture.

Beschrijving van de resultaten:

A method for the simultaneous determination of soil moisture and soil density in a soil column by γ -attenuation has been worked out.

Principle

Gamma radiation which passes a layer of wet soil is attenuated according to

$$I = I_0 \cdot e^{-(\mu_s \cdot \bar{\rho}_d + \mu_w \cdot \theta)}$$

in which

I_0 = countrate without attenuation

I = countrate after attenuation

$\bar{\rho}_d$ = dry soil bulk density (g per cm^3 bulk soil)

θ = soil moisture content (g per cm^3 bulk soil)

μ_s = attenuation coefficient due to soil

μ_w = attenuation coefficient due to water.

Both μ_s and μ_w are a function of the energy of the radiation. In other words, μ_s and μ_w are different for the 0.66 MeV radiation of Cesium-137 and the 0.06 MeV radiation of Americium-241.

The simultaneous determination of $\bar{\rho}_d$ and θ is based on this difference. To determine their values, both the attenuation for Am and Cs radiation are measured at the same time. Working out the mathematics leaves 2 equations with $\bar{\rho}_d$ and θ as the 2 unknowns, which can be solved easily.

Instrument

Although the principles for a double beam instrument to determine water and density simultaneously are already known for some years (Gardner et al 1967), the practical difficulties were considerable and have delayed its realization. For details concerning the instrument, see report of project 33.

Experiments with swelling soil

An experiment with a heavy clay soil was conducted. A counting time of 1 min. is preferable. Due to the nearby Cs source a fixed correction of 9.7 c.p.s. must be subtracted from the Am count rate. All countings are corrected for a 4.75 μ s dead time for the Am circuit and with a 6.50

μ s dead time for the Cs circuit. The determined attenuation coefficients for water are $0.2006 \text{ cm}^2 \text{ g}^{-1}$ for Am and $0.08515 \text{ cm}^2 \text{ g}^{-1}$ for Cs. The following attenuation coefficient values for the soil were found: $0.07761 \text{ cm}^2 \text{ g}^{-1}$ for Cs and $0.2965 \text{ cm}^2 \text{ g}^{-1}$ for Am. These soil attenuation values differ markedly with the parent material of the soil, e.g. for sand attenuation values of $0.07612 \text{ cm}^2 \text{ g}^{-1}$ for Cs and $0.2403 \text{ cm}^2 \text{ g}^{-1}$ for Am were determined. These large differences will severely limit the use of natural undisturbed soil samples with unknown parent material or with strongly varying material with depth.

When an initially dry clay soil is brought into equilibrium, with a groundwater level, swelling arises and hence a change in moisture content as well as in bulk density occurs. In an illustrative figure 1 the initial (i) and the equilibrium (f) situation is given in the parameters void ratio (e) and moisture ratio (v). Since the overburden potential had to be taken into account in the description of equilibrium in swelling soils it can be shown that the pressure equivalence of the moisture potential increases with height. This is opposite to the trajectory of this potential in an equilibrium state in a non-swelling soil and explains why the moisture content increases with height in a swelling soil.

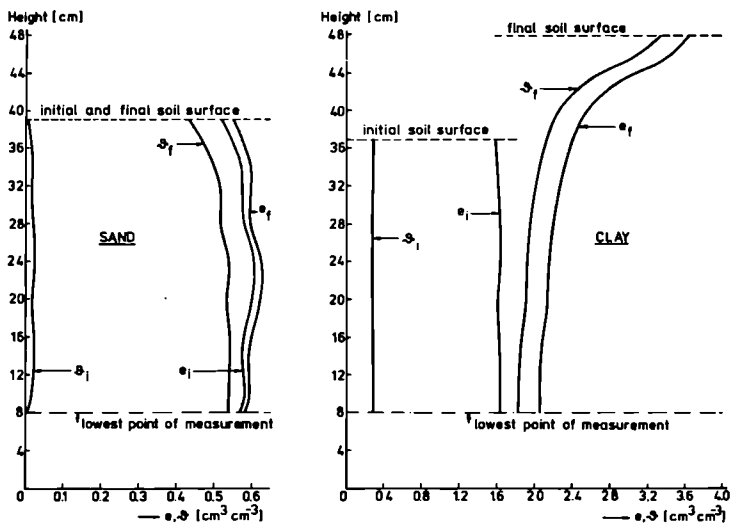


Figure 1. The void ratio (e) and the moisture ratio (v) in relation to height and time in a swelling clay soil. Comparison with a sandy soil.

Resultaten van het project No. 3

Hoofd van het team en wetenschappelijke medewerkers:

F. van Dorp, M.J. Frissel, P. Poelstra, J. Sinnaeve.

Titel van het project: Ionic composition of the soil solution, as a function of time and place, with respect to the ion uptake by plant roots.

Beschrijving van de resultaten:

Simulation Model

A CSMP simulation model of one root in a soil cylinder was worked out. The model describes the adsorption of nutrient ions and water separately. Water and ion uptake respectively depend on the water tension in the soil and the ionic concentration in the soil solution, both in the root hair zone. The root is divided into several segments with different uptake activities for water and ions. Considered is also the fact that plants do not absorb the same amount of water and ions during day and night.

The soil contains water, nutrients in solution, nutrients adsorbed on the exchange complex of the soil e.g. potassium, and nutrients in solid phase e.g. phosphorus. Between the nutrients in solution and, on the one hand, those adsorbed on the complex, on the other hand those in the solid phase, respectively instantaneous and non-instantaneous equilibria exist.

The uptake of potassium and of phosphate were simulated with data from Kauffman and Bouldin and from Van Diest (Tables 1 and 2).

The major conclusion from these simulations is, that more information is needed about the influence of different physical and chemical situations on the root growth and on the root uptake activity. This will be investigated in cooperation with project 6.

Hybrid simulation, i.e. simulation with a combined digital and analog computer.

Water transport through the soil, as compared to other processes, is a very rapid process. Simulation of such a process exceeds the possibilities of a digital computer. Models are actually made in the University of Gent by Prof. Vansteenkiste and co-workers in cooperation with the Dept. of Theoretical Crop Production of the

Agric. University of Wageningen and the Association Euratom-ITAL, Wageningen to simulate water movement in soil with an hybrid computer.

Cation Exchange

In view of simulating transport of cations through soils Frere, Frissel, and Reiniger made a computer program, called CATEX, for the exchange of four cations, between the soil cation adsorption complex and the soil solution. The exchange equations based on the Eriksson equation were calculated with Newton's iteration method. As more data are available for the Gapon exchange equation, a comparison between CATEX and the Gapon approach was made. In a large range of concentrations, results obtained with both equations are similar, when it is accepted that the Vanselow constant is equal to 2 times the cation exchange capacity multiplied by the square of the Gapon constant.

Table 1: Simulated uptake of potassium.

TOTINT	RSAS	UPTS	TUPTS
0.02	1	0.5×10^{-5}	2
0.02	10	4.9×10^{-5}	19
0.02	50	21.6×10^{-5}	84
0.002	10	0.5×10^{-5}	2
0.002	50	2.2×10^{-5}	8

TOTINT = total initial conc. of potassium (meq/cm³ soil)
 RSAS = root segment uptake activity of the first 10 segments (cm/day)
 UPTS = total uptake by a root segment of 1 cm length (meq/cm root)
 TUPTS = total potassium uptake by a plant with 10⁴ cm roots after one season (mg K).

Table 2: Simulated uptake of phosphate

SOLINT	ADSINT	DS	RATEF	RSAS	UPTS	TUPTS
0.005	0.2	0.4	0.15	1	1.8×10^{-3}	555.
0.005	0.05	0.4	0.05	5	5.4×10^{-4}	166.
0.001	0.05	0.4	0.01	1	3.1×10^{-5}	9.6
0.001	0.05	0.4	0	1	2.1×10^{-5}	6.5
0.001	0.05	0	0.01	1	1.3×10^{-5}	4.0
0.001	0.05	0	0	1	0.7×10^{-5}	2.3

- SOLINT = initial concentration solved $H_2PO_4^-$ (meq/ml solution)
 ADSINT = initial concentration solid phosphate (meq/cm² soil)
 DS = diffusion constant of $H_2PO_4^-$ in free water (cm²/day)
 RATEF = rate factor for realizing equilibrium.
 UPTS = total uptake by a root segment of 1 cm length (meq $H_2PO_4^-$ /cm root)
 TUPTS = total phosphorus uptake by a plant with 10⁴ cm roots after one season (mg P).

Publication : Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project No. 4.

Hoofd van het team en wetenschappelijke medewerkers:

J.P. Rolland, M.J. Frissel, P. Poelstra.

Titel van het project: Transport of pesticides (insecticides, fungicides) and herbicides in soils.

Beschrijving van de resultaten:

Of the physical processes influencing the fate and behaviour of biocides in soils, adsorption is the most important; this adsorption may modify the physical distribution, the biological activity and even the susceptibility of the biocide to microbial metabolism. The adsorption is mainly affected by, of course, the characteristics of the biocide, by the organic matter content of the soil and in some cases, by the pH and clay content.

The physical transport of biocides in the soil occurs by leaching, volatilization and runoff; it depends on such soil characteristics as structure, moisture content, temperature, pH, etc. The microbial metabolism is mainly responsible for their degradation; persistence is, of course the final result of the interaction of all these processes. An insecticide Trichloronate ($C_{10}H_{12}Cl_3O_2PS$), was sprayed ($50 \mu\text{gr}/\text{cm}^2$) on the surface of two soil (9% organic matter) columns; artificial rainfall ($26,5 \text{ mm H}_2\text{O}/\text{Day}$) was applied at the top of the column during 32 days. Effluent samples were taken and, after extraction with ethylacetate, Trichloronate residues in these effluents and in the soil were measured by a gas chromatograph electron capture detector at 200°C . In this soil almost the total amount (in some cases up to 99,8%) of the applied compound was adsorbed in the uppermost layer. No Trichloronate was detected in the effluents and only traces were present at 3 cm depth in the column (fig. 1a and 1b). Considering its half life of 60 days in this soil and its adsorption in the surface layer, the Trichloronate insecticide may cause unwanted side effects.

The herbicide, Bromacil ($C_9H_{13}O_2N_2Br$) was sprayed ($26 \mu\text{gr}/\text{cm}^2$) respectively on a sandy humic (3 - 1% organic matter) soil and on a river loam (1% organic matter) soil. Artificial rain was applied for 50 days ($26.5 \text{ mm H}_2\text{O}/\text{day}$); nearly the total amount of the spray passed through the 30 cm column with a peak of 0.4 ppm in the effluent of the river loam on the 19th day and a maximum of 0.5 ppm in the sandy humic soil on

the 30th day (fig. 2). Only traces were retained by both soils. Bromacil appears to be rather mobile and therefore less dangerous as a surface pollutant but according to its half life of 5 to 6 months, may eventually contaminate the ground water.

A simulation model written in CSMP was worked out and used in a description of the behaviour of Bromacil on the same soil, varying some parameters. In this model, the soil was divided in numerous layers (0.5 to 3 cm of thickness) and small (0.1 day) time-steps were considered. Furthermore it was accepted that the equilibrium between solid and liquid phase is instantaneous, that the adsorption isotherm is linear and that convection and diffusion occur successively.

This first model gave valid information on the movement of Bromacil, shown by the comparison of the general shape of the theoretical (fig. 3 and 4) and experimental (fig. 2) curves; more factors and parameters should be introduced in the final model to control the fate of pesticides in soils.

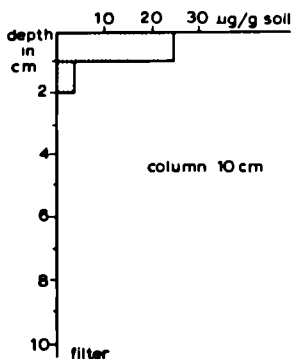


Fig. 1a

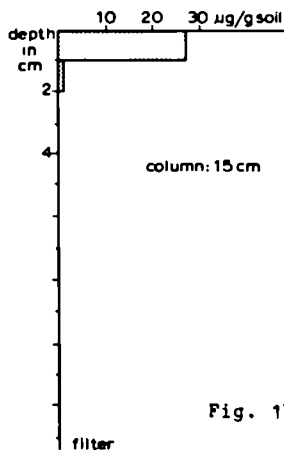


Fig. 1b

Fig. 1a - Trichloronate ($50 \mu\text{gr}/\text{cm}^2$) adsorbed in relation to depth after 32 days of artificial rain ($848 \text{ mm H}_2\text{O}$) (column of 10 mm length).

Fig. 1b - Trichloronate ($50 \mu\text{gr}/\text{cm}^2$) adsorbed in relation to depth after 32 days of artificial rain ($848 \text{ mm H}_2\text{O}$) (column of 15 cm length)

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

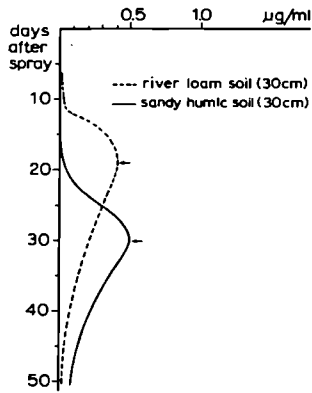


Fig. 2 - Bromacil effluents in relation to time after spray (26 $\mu\text{gr/cm}^2$) application. Artificial rain during 50 days (1325 mm H_2O).

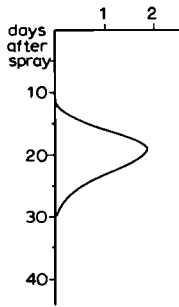


Fig. 3 - Theoretical curve representing Bromacil movement in soil. Convection considered in the simulation model.

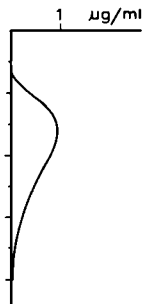


Fig. 4 - Theoretical curve representing Bromacil movement in soil. Convection and diffusion considered in the simulation model.

Hoofd van het team en wetenschappelijke medewerkers:

J.H. Becking.

Titel van het project: Chemistry and biology of nitrogen in soils.

Beschrijving van de resultaten:

The work accomplished on this project in 1972 covers three main topics, which, however, are interrelated with each other.

I. Transformation of nitrogen in a water-soil system.

II. Ecology of rice soils in relation to nitrogen.

III. Eutrophication of water.

I. Transformations of nitrogen in a water-soil system

An investigation of such a system is important for a study of the nitrogen uptake and efficiency in rice soils and for the study of eutrophication of water. A model system was used and the methodology was tested out. In the model system being a vessel with a closed atmosphere, two soils were tested, a tropical soil and a clay soil of the Netherlands. N-15 labelled urea (31 % excess N¹⁵) and KNO₃ (48 % excess N¹⁵) was added to the soils. Gaseous nitrogen compounds produced from these dressings were analysed and their N-15 isotope content determined by a combination of gas chromatography and mass spectrometry. To simulate natural conditions the soils were temporarily drained and then flooded again. The total system was incubated in the light at 28 - 29°C (35,000 lux, 12 light and 12 dark hours), which allowed the development of a profuse algae growth in the water covering the soil. In a duplicate experiment rice plants were grown in similar conditions on these soils. To facilitate gas determinations in the closed system an artificial gas mixture of 80 vol. % He and 20 vol. % O₂ was used. As experimental outcome can be given that the O₂ level in the closed atmosphere decreased in 10 - 12 days from 20 vol. % to 2 - 6 vol. % O₂, whereas the CO₂ and N₂ concentrations increased to 1 - 2 vol. % and 2 - 15 vol. %, respectively. Finally an equilibrium was reached at the 5 vol. % O₂ level, due to photosynthetic O₂ production and O₂ consumption in the system. In the alkaline Dutch soil as much as 14 % of the urea dressing was recovered as ammonia in the atmosphere and this was only 5.5 % when the soil was not

water-lodged. Although the tropical soil had a pH of 6.5 - 7.0, no ammonia was recovered in the atmosphere suggesting an ammonia fixation to the soil. In water-lodge conditions primary CH_4 was produced and 4 - 5 % of the urea or nitrate gift was recovered as N_2 in the atmosphere. In the rice plants about 25 - 30 % of the nitrogen dressing was recovered in the upper parts of the plants.

II. Ecology of rice soils in relation to nitrogen

By a grant of the Netherlands Foundation for the Advancement of Tropical Research (WOTRO) a field study of the nitrogen ecology in rice soil could be made. The experiments were conducted at the Muara Experimental Station for Rice Culture, near Bogor in Java, Indonesia. They cover a period of 2,5 months in the rain season. As method for the assessment of nitrogen fixation in the field the acetylene reduction test was used. In this test acetylene functions as analogon for nitrogen in the enzyme nitrogenase. No nitrogen fixation could be measured by heterotrophic nitrogen-fixing bacteria (Beijerinckia and Azotobacter) in these soils. The contribution of nitrogen to the soil by free-living blue-green algae having the capacity of fixing nitrogen (figure 1) was low, because sparse distribution of these algae in these soils, being only abundant locally. Important was the discovery that the aquatic fern Azolla pinnata R. Br, which possesses a symbiosis with blue-green algae within its leaves, can contribute appreciably to the nitrogen economy of these soils. An Azolla cover of 100 % of the water surface fixed an amount of 335 - 670 kg N per ha annually. A study of the biological nitrogen cycle in these soils is important, because it gives insight in the processes going on and it will provide means to anticipate eutrophication of these waters. Eutrophication of the water will readily occur in rice soils by overdressing.

III. Eutrophication of water

Eutrophication of the aquatic system has become intensified in many areas by excessive dressing of agriculture soils and by human inhabitation, due to sewage production. In the study undertaken the emphasis is particularly on the role of nitrogen and phosphorus in this eutrophication process. Eutrophication occurs when there is an excess of organic and inorganic compounds in the water and

eutrophication may be partly due to algae blooms in the water. In many eutrophic waters blue-green algae, many of them fixing nitrogen, are the dominant bloom forming species. Thus, even when it is possible to remove completely the input of combined nitrogen into fresh water habitats, blooms of nitrogen-fixing algae (Anabaena, Nostoc, etc.) will occur when phosphorus and other nutrients are available. So far there is strong indication that in temperate regions phosphorus is the limiting factor for bloom formation in algae and that the phosphorus load of surface water is primarily caused by urban effluents. In these effluents particularly polyphosphates used in commercial soaps and detergents seem to be important. Experiments were performed to test the growth of blue-green algae (Nostoc sp.) on various phosphorus sources such as ortho-, meta-, and polyphosphate. Polyphosphate is not readily assimilated by blue-green algae and it seems that high concentrations (180 mg P/L) are toxic for growth, but after prolonged incubation it can be used as phosphorus source in metabolism (Table 1). Further experiments will be conducted on the natural phosphorus level in the fresh water of urban effluents and on the algal composition of these eutrophic waters.

Publications:

J.H. Becking. Oecologisch-hydrobiologisch onderzoek van natte rijstvelden in verband met vastlegging van atmosferische stikstof door deze grond.

Report Netherlands Foundation for the Advancement of Tropical Research (WOTRO), 42 pp (1972).

J.H. Becking. Symbiosen: Stickstoff-Bindung.
Fortschritte der Botanik 34, 459-467 (1972).

Annual report 1972 of the Association EURATOM-ITAL.

Table 1

Nitrogen yield of cells grown in 100-ml medium (see note) containing all nutrients except phosphorus, *Nostoc* sp. Harvest after growth for 16 and 32 days at 28°C and light intensity of 35,000 lux.

P-source	Incubation period	
	16 days	32 days
No added phosphate	0.71	1.09
Orthophosphate (K_2HPO_4)	2.02	3.21
Methaphosphate	1.23	1.52
Polyphosphate	0.49	2.75

Note: Phosphorus concentration: 180 mg P/litre
 Medium $MgSO_4 \cdot 7H_2O$ 0.15 g, $CaCl_2$ 0.05 g, NaCl 0.05 g,
 Fe-citrate 0.01 g, citric acid 0.01 g, distilled water
 1000 ml (pH = 7.0).

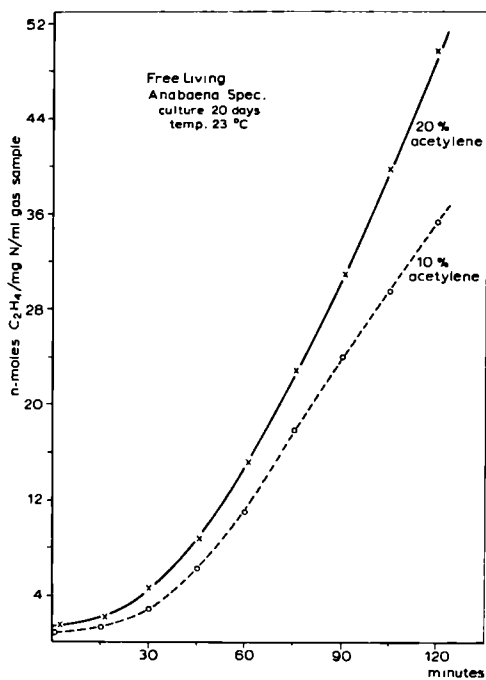


Fig. 1. Acetylene reduction by free-living *Anabaena* spec. (Cyanophyceae) in a nitrogen-free culture medium at two levels of acetylene gas (10 and 20%). The figures are given in n-moles C_2H_4 per mg N per ml gas sample.

Resultaten van het project - No. 6.

Hoofd van het team en wetenschappelijke medewerkers:

J. Simnaeve, F. van Dorp, M.J. Frissel.

Titel van het project: Ion uptake by intact plants from the soil solution or from the equilibrium solution of a synthetic resin. Experimental and simulation model approach.

Beschrijving van de resultaten:

The principal aim of this project, started in September 1972, is the study of the influence of an adsorption complex, assuring a subsequent delivery of nutritive elements, upon the absorption of ions by a living plant. This aspect within the sequence of simultaneous processes leading to the physiological absorption of an ion has hitherto been somewhat neglected as most of the experimental data reported in the literature have been obtained with solution cultures.

The following aspects of this problem will be studied and some preliminary experiments were started concerning:

- Ion absorption by intact living plants from carefully selected clays or synthetic resins, formed by the copolymerization of styrene and divinylbenzene with attached ionizable groups which determine their chemical behaviour, at varying ground water level.
- Influence of the cation exchange capacity of the substrates varied by an appropriate choice of resins and clays or by their mixtures with sand.
- Changes induced by the presence of the plant in its physico-chemical environment. These changes are intimately bound to the abundance of the root system and the rooting configuration. For this reason, monocotyledonous as well as dicotyledonous plants will be used.
- Variation of the ionic composition of the soil solution around the absorbing root surfaces in relation to time and place. This variation is the major problem in the study of the kinetics of the absorption process. In this respect, collaboration within projects 3 and 6 is of considerable importance.
- Improvement of the existing simulation models with emphasis on experimental checking of some of the parameters directly related to the activity of the root system. Two models actually available will be used as a working base. The first one, by Frissel et al applies to a complex geometric root system in solution culture; the second one,

by Van Dorp (see project 3), describes water and ion fluxes in a soil core during absorption by one cylindrical root.

Actually, the study of the literature is in good progress and a special device to study the influence of the ground water level on the root system is in development. A nutrient medium, using Dowex 50 W (a strong acid cation exchanger with nuclear sulfonic acid as active group and a total exchange capacity of 1.9 meq per ml wet resin) and Dowex 21 K (a strong base anion exchanger with trimethyl benzyl ammonium as active group and a total exchange capacity of 1.25 meq per ml wet resin) is in preparation. Some preliminary results indicate that the resins can be charged by treating them with a Hoagland solution (Hoagland - Arnon I solution with 20 meq per liter). The cationic charge of the Dowex 50 W resin is 82% Ca, 14% Mg and 4% K. The anionic charge of the Dowex 21 K is actually investigated.

Publication :

Annual report 1972 of the Association Euratom-ITAL.

Resultaten van het project No. 7

Hoofd van het team en wetenschappelijke medewerkers:

G. Verfaillie.

Titel van het project: Study of the Kinetics of Ion-uptake by intact Plants.

Beschrijving van de resultaten:

1. Aims

It is usually assumed that the balance between the cation- and anion-uptake by plants is realized by the hydrogen ions. In order to verify this assumption, the kinetics of total acid-uptake (free hydrogen ions and bound protons) has been studied in relation to the phosphate ion-uptake and to the presence of the other major components of the nutrient solution.

2. The experiments

23 kinetic runs have been performed with batches of 24 six weeks old rice plants (Oryza sativa L.cv Arborio). Using the closed system technique and the experimental set-up described in earlier reports, the uptakes of phosphate and of acid have been followed simultaneously and continuously during about 2 days. The role played by the uptake of various ions in the acid-uptake has been put in evidence by comparing the acid-uptake from various combinations of ionic components of an Hoagland-Arnon I nutrient solution. Before starting each kinetic run, the nutrient solution had been acidified to pH 3.5 with HNO_3 .

3. Results and comments

In fig. 1, the rates of acid-uptake from various combinations of ionic solutions are plotted against the pH at which the acid has been taken up.

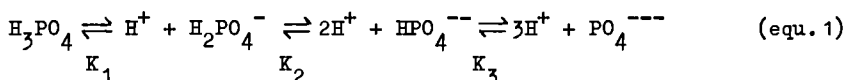
- Curve H corresponds to a full Hoagland-Arnon I solution, curve M to the the same solution without phosphate (Matrix solution) and curve P to an acidified $1 \text{ mM}/1 \text{ KH}_2\text{PO}_4$ solution (H without M). In all these treatments, the mineral nutrition process produces an increase of the pH, as it is indicated by the arrows along the curves.
- Below pH 4.65, the acid-uptake proceeds at nearly the same rate

in solution H and in solution M. Above pH 4.65, the presence of the phosphate enhances merely the acid-uptake with a maximum effect around pH 6. The fraction of the acid-uptake due to the phosphate-uptake alone is represented by the shaded area.

- When the uptake proceeds in a KH_2PO_4 solution acidified with HNO_3 , in the absence of any other matrix (curve P), the pH increases slowly but stabilizes itself at a value somewhat smaller than 4.65. If, when the pH is stabilized, various combinations of ionic components of the matrix are injected into the nutrient solution, nitrates, and nitrates only, produce a further increase of the pH above the threshold value, independently of the nature of the associated cation. For an injected amount of nitrate equal to that existing in a full H solution (15 meq/l), the resulting acid-uptake (curve H') proceeds in the same way as in the full H solution.
- To the contrary, injection of sulfates produces a slow but continuous decrease of the pH.

4. Theoretical interpretation. The phosphate - hydrogen binary system

Considering the equilibria,



the uptake of one specific phosphate form, containing n bound protons, produces a variation of acidity in the nutrient solution expressed by:

$$\frac{d[\text{H}^+]}{d^c} = \frac{\alpha_3 - \alpha_1 + n - 2}{1 - \frac{K_w}{[\text{H}^+]^2} + C \frac{d(\alpha_1 - \alpha_3)}{d[\text{H}^+]}} \quad (\text{equ. 2})$$

where C is the total phosphate concentration, α_1 and α_3 are the fractional concentrations of the H_3PO_4 and HPO_4^{--} forms respectively.

The experimental results can be summarized by:

$$\frac{d[\text{H}^+]}{d^c} > 0 \text{ for } \text{pH} > 4.65 \quad (\text{equ. 3})$$

Owing to this, and the denominator of the right hand member of

equ. 2 being positive for all pH value smaller than 7, equ. 2 reduces to:

$$\frac{d[H^+]}{d^c} > 0 \text{ for } \alpha_3 - \alpha_1 > 2 - n \quad (\text{equ. 4})$$

This condition can be fulfilled only when n is equal to 2.

Consequently, the only phosphate form taken up is $H_2PO_4^-$.

The threshold pH is that at which α_1 and α_3 are equal. Thus, at the threshold pH we have:

$$[H^+]^2 = K_1 K_2$$

$$\text{or } \text{pH} = 0.5 \text{ p}K_1 K_2 = 4.66$$

The hypothesis of the phosphate-hydrogen binary system, based on the experimental data, is confirmed by the theory.

A similar interpretation based on the ionic dissociation of the sulfuric acid leads to the conclusion that the sulfate-uptake is due to the SO_4^{--} ion only and produces a slight but continuous release of acid as it was observed during some of the experiments.

5. Conclusions

- The consumption of hydrogen ions during mineral nutrition of the plant in a solution of the Hoagland-Arnon I type is not the simple result of the charge balance between cation- and anion-uptakes but is merely a necessary process linked to the anion-uptake.

- The acid-uptake is the resultant of 2 main combined effects:

The uptake of NO_3^- requires a stoichiometric consumption of H^+ or release of OH^- , in the full range of pH.

Displacing the ionic dissociation equilibria of the corresponding polyprotic acid, the uptakes of $H_2PO_4^-$ and of SO_4^{--} require respectively an acid consumption when the pH lies between 4.66 and 7 and an acid release at low pH.

Publications on the subject in 1972:

G. Verfaillie. A Method for the Study of the Kinetics of Photosynthesis at Constant Rate of Transpiration. Results obtained with rice plants (Oryza sativa L. cv Arborio). Journal of Experimental Botany, 77, pp 1106-19, Nov. 1972.

G. Verfaillie. A Method for the Study of the Kinetics of Ion-Exchanges by Intact Plants under strictly Controlled Conditions. Results obtained with phosphate ions and rice plants (Oryza sativa

L., cv Arborio).

Journal of Experimental Botany. (to be issued in 1973).

Annual report 1972 of the Association EURATOM-ITAL.

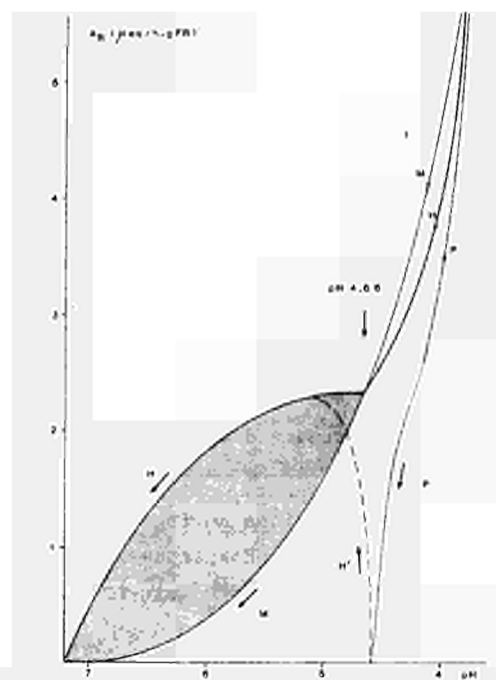


Fig. 1 The influence of the pH and of the composition of the nutrient solution on the rate of acid-uptake.

A_R = total acid-uptake rate (micro-equivalent per hour and per gram fresh root).

H = full Hoagland-Arnon I nutrient solution.

M = matrix solution (KH_2PO_4 - free H solution).

P = 1 mM/l KH_2PO_4 solution acidified with HNO_3 .

H' = after injection of 15 mM/l nitrate in P solution.

The arrows along the curves indicate the direction of the pH scanning during the runs.

Resultaten van het project No. 8.

Hoofd van het team en wetenschappelijke medewerkers:

C. Petit, M. Karim, J. Sinnaeve, M.J. Frissel, A. Ringoet.

Titel van het project: Ion uptake by roots from dilute solution.

Beschrijving van de resultaten:

A. Phosphate uptake by soybean (*Glycine max* L. (Merr.))

A considerable phosphate-accumulation capacity of the soybean stem was shown "in vivo", last year, by the use of ^{32}P and semi-conductor detectors. The previous report suggested that this accumulation was really metabolic and that the exchange of ^{31}P -ions for ^{32}P , if it occurred, must be small as compared to the first process. In order to verify this hypothesis, the influence of metabolic factors on phosphate uptake from nutrient solutions (range of KH_2PO_4 concentrations from 0,32 up to 963 μM) is studied. The following aspects are considered:

- light effect on the phosphate accumulation in the stem and total uptake by the plant.
- temperature effect on the stem phosphate accumulation.
- comparison of the phosphate accumulation rate respectively in the roots, the stem, the leaves with petioles.

The plants are 21 days old and have grown, since germination, on the experimental phosphate concentration, maintained at constant level. The semiconductor detectors method, liquid scintillation counting, both in combination with the continuous flow technique (see previous report) were used in these experiments.

Light effect

A higher light intensity increases the ^{32}P accumulation rate of the stem (fig. 1) as well as the total uptake rate by the plant (fig. 2), especially at concentrations higher than 3,2 μM KH_2PO_4 . It is known that the controversy about light effect in the literature is also related to phosphate concentrations.

Phosphorus-32 is mainly accumulated in the lowest internode of the stem when the uptake period of the radioactive solution is short (2 min.) and the experiment is performed during the first three hours light (28.000 lux \pm 1000) after darkness.

The same experiment, starting later during the day (after 6 hours of light) shows a larger accumulation in the top of the stem.

If the uptake period is prolonged to 80 min. and the experiment is done during the first three hours of light or during the first three hours of darkness, ^{32}P is mainly accumulated in the top of the stem. During the night, however, the process is slower. Accumulation of ^{32}P in three consecutive internodes along the stem presents a gradient shape; the lowest internode, just above the cotyledons, has the smallest accumulation rate. At higher light intensities, the gradient apparently disappears (fig. 3): the accumulation rate along the stem becomes uniform.

These observations may lead to the following interpretation:

- Phosphorus-32 accumulation in the stem, after root absorption, depends upon the amount of carbohydrates produced in the aerial part.
- Substances, recently produced by photosynthesis, probably play an important role in the accumulation process.
- When the photosynthetic products are limiting, they mainly show their effect in the younger tissues of the plant.

Temperature effect

Temperature variation from 18 °C to 28 °C in the surroundings of the plant increases the ^{32}P accumulation rate in the stem (fig. 1). The effect, however, is not observed in darkness or at low phosphate concentrations in the nutrient solution ($\leq 3,2 \mu\text{M KH}_2\text{PO}_4$). This result suggests again a relation between carbohydrates and phosphorus accumulation in the stem, if it is accepted that temperature modifies the photosynthetic efficiency as well as the rate of metabolic uptake by root tissues.

One preliminary experiment was performed in order to precise whether temperature variation in the root medium or in the surroundings of the aerial part is more efficient with regard to ^{32}P -accumulation in the stem. When the photosynthetic rate is high (42.000 lux) and the nutrient solution contains $32 \mu\text{M KH}_2\text{PO}_4$, a temperature variation from 18 °C up to 28 °C around the root system causes a larger (approx. 2 times) increase of the ^{32}P -accumulation than does a similar variation in the leaves surroundings (fig. 4).

Accumulation of phosphate in different parts of the soybean plant.

The phosphate accumulation kinetics expressed per unit weight of stem and leaves are very similar, although both tissues have entirely different functions and structure. This similarity is illustrated by the affinity

(K_m) and the capacity (V_m) (data in table 1 and fig. 5). The amount of tissue produced in leaves is twice as high as in the stem (fig. 6 and 7). Therefore, the conclusion is that the phosphate accumulation is essentially metabolic, but that its utilization in the stem is not strictly related to the tissue production "in situ".

Comparing the response of yield and accumulation rate curves to different phosphate concentrations in the stem and the leaves, it is observed that the yield curve reaches its maximum at a lower concentration than the accumulation curve (fig. 6 and 7). This observation, particularly clear for the stem, suggests a more important luxury consumption in this plant part than in the leaves. The phosphate redistribution from the stem (see phosphorus-carence data in previous report) also suggests that there is a real phosphate stockage (sink) in the stem.

The saturation kinetics of the root system is slower than that of the aerial part (fig. 5). This is the cause of the slow saturation of the total uptake isotherm, as published by some authors, working with radioactive isotopes.

The phosphorus distribution in the plant is affected by a concentration effect. At low concentration ($< 16,05 \mu\text{M KH}_2\text{PO}_4$) the root system keeps the greatest part of absorbed phosphorus (fig. 8), and this results in a decrease of the growth of the aerial part (fig. 6 and 7). The inversion in the phosphate distribution (fig. 8) occurs in a concentration range, where, according to the literature, the second mechanism of the Michaelis-Menten kinetics starts and the microorganisms have no effect on the uptake.

Table 1 - Michaelis-Menten constants with standard deviation calculated by Cleland program. V_m is expressed in $\mu\text{g}\cdot\text{h}^{-1}\cdot\text{g}^{-1}$ dry weight of tissue and K_m in M.

	root system	foliar system	stem
V_m	166 ± 12	37 ± 3	33 ± 1
K_m	$(0,34 \pm 0,08) 10^{-4}$	$(0,15 \pm 0,04) 10^{-4}$	$(0,15 \pm 0,03) 10^{-4}$

B. Double labelling of ion-uptake experiments

A detailed proposal for a double labelling experiment to be carried out in different laboratories, members of the European Society of Nuclear methods in Agriculture (ESNA), working group 5 "Nuclear techniques in the study of soil-plant relationships", has been worked out. Absorption experiments with

intact tomato plant (*Lycopersicon esculentum* L. cv. Murette), using Cesium (^{134}Cs , ^{137}Cs) as an element not yet present in the biological tissue and calcium (^{45}Ca , ^{47}Ca) as an element already present, are in progress.

Publications:

C. Petit. Accumulation du Phosphore dans les différents organes de la plante de Soja (*Glycine max*, L (Merr.)). Mémoire fin d'études, Louvain, 1972.

Annual Report ESNA-meeting, Budapest, 26-29 September 1972.

Annual report 1972 of the Association EURATOM-ITAL.

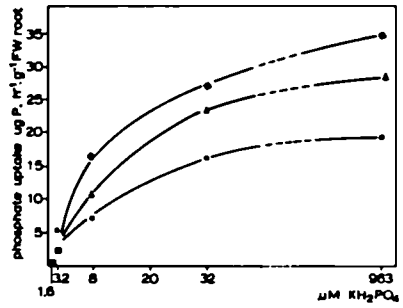
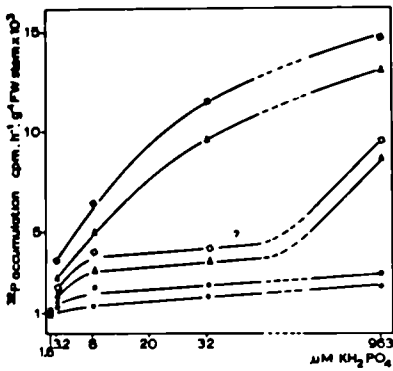


Fig. 1 and 2 - Effect of light intensities on the rate of ^{32}P accumulation by the stem (fig. 1) and on total phosphate uptake by the plant (fig. 2), at different concentrations in the nutrient solution. Closed symbols: 28°C temperature around root and aerial part. Open symbols: 18°C temperature around root and aerial part. \bullet, \circ : 0 lux; $\blacktriangle, \triangle$: 26.000 lux; \blacklozenge, \lozenge : 42.000 lux.

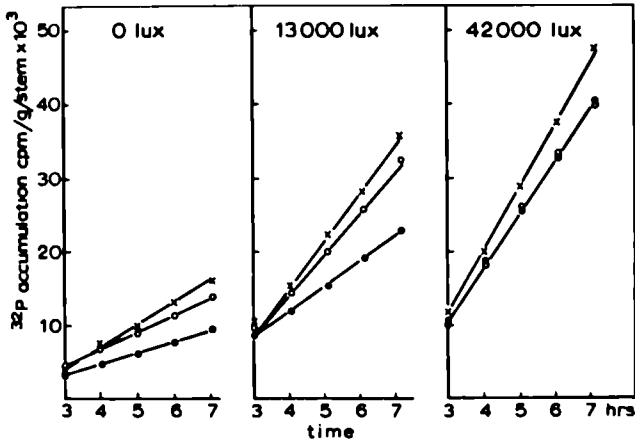


Fig. 3 - Rate of ^{32}P accumulation by consecutive internodes of the stem at different light intensities: lowest \bullet , middle \circ , highest \circ .

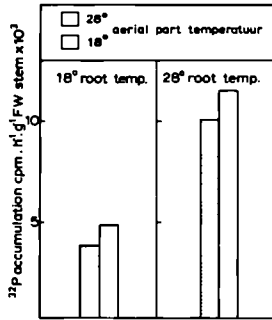


Fig. 4 - Effect of variable "aerial" temperature at two levels of root temperature (18°C - 28°C) on the rate of ³²P-accumulation by the stem (42.000 lux light intensity and 32 μM KH₂PO₄)

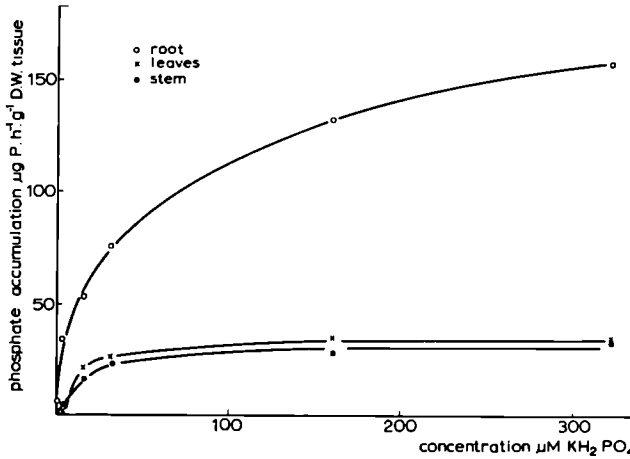


Fig. 5 - Phosphate accumulation rate per unit of dry weight of different plant parts in relation to the following concentrations in the nutrient solution: 0,321; 3,21; 16,05; 32,1; 160,5; 321 μM KH₂PO₄.

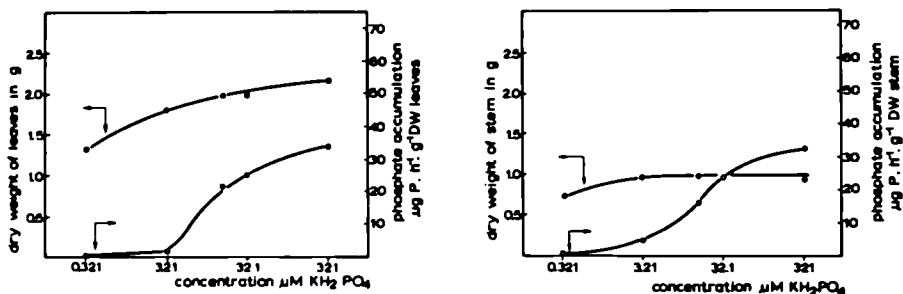


Fig. 6 and 7 - Yield in dry matter (+) and accumulation rate of phosphate (o) for the leaves (fig. 6) and for the stem (fig. 7) in relation to the following concentrations (semi-log. scale) in the nutrient solution : 0,321; 3,21; 16,0; 32,1; 321 ($\mu\text{M KH}_2\text{PO}_4$).

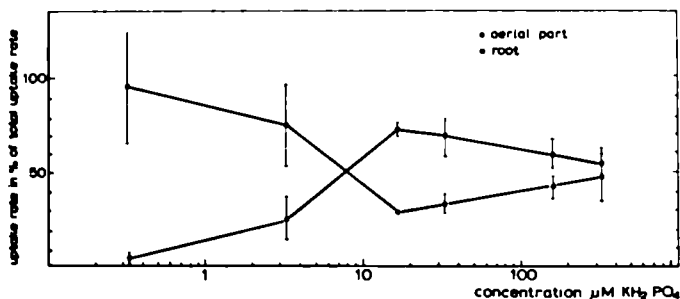


Fig. 8 - Repartition of phosphate absorbed per hour between the aerial part, and the roots, in relation to the following concentrations (semi-log. scale) in the nutrient solution: 0,321; 3,21; 16,05; 32,1; 160,5; 321 ($\mu\text{M KH}_2\text{PO}_4$).

Resultaten van het project No. 9.

Hoofd van het team en wetenschappelijke medewerkers:

G. Desmet, A. de Ruyter, A. Ringoet, G. Sauer.

Titel van het project: Uptake and release of ions by subcellular structures, mainly chloroplasts and mitochondria.

Beschrijving van de resultaten:

Research in this project, which is directly related to microlocalization problems in project 30/75, was limited to some aspects of the Ca^{++} uptake by chloroplasts isolated from spinach (*Spinacea oleracea* L., cv. Noorman). Spinach plants are grown for 4 weeks in a climate-controlled chamber on a complete nutrient solution. The isolation method is similar to the one proposed by Nobel and yields 70% class I chloroplasts. Calcium content, uptake and release in the incubation medium and in the chloroplasts was measured by ^{45}Ca -tracing and by atomic absorption spectrophotometry.

A. In a first group of experiments factors immediately affecting the Ca^{++} -content of the chloroplasts were studied. Contrary to what has been stated in an earlier report, the Ca-content of the chloroplasts varies between 20 and 35 $\mu\text{g Ca}^{++}$ per mg chlorophyll. Temperature of the incubation medium, varying from 0° up to 30° C, does not affect the calcium content. A decrease is observed at higher temperatures, probably due to denaturation of the structure of the organelles (Fig. 1).

Between the Ca-content of the chloroplasts and the pH of the medium a negative linear relationship exists (Fig. 2). The chloroplast concentration of the incubation medium (range: 47-236 $\mu\text{g chlorophyll/ml}$) does not influence the calcium content per unit chlorophyll (average value: 2.29 $\mu\text{g Ca}/100 \mu\text{g chlorophyll}$), when no external Ca^{++} is supplied.

B. In a second group of experiments calcium uptake, immediately after isolation and at varying concentrations of the incubation medium was measured. Results in fig. 3 show a saturation type of curve with a maximum reached at approx. $5 \cdot 10^{-3}$ M Ca^{++} . The maximum uptake is 25 - 30 $\mu\text{g Ca}/100 \mu\text{g chlorophyll}$ at 20°C and the calculated V_{max} at this temperature equals 32 $\mu\text{g Ca}^{++}/100 \mu\text{g chlorophyll/min}$.

Data in Table 1 show that calcium taken up from incubation media of increasing concentration reaches different compartments in the chloroplasts; at higher concentrations relatively more calcium is bound and not watersoluble anymore.

Such external factors as temperature (range: $0-30^{\circ}\text{C}$) and pH (6 up to 9) have a limited influence on calcium uptake in the present experimental conditions. A non-linear, mainly S-shaped relation, is found between Ca-

uptake, at different external concentrations, and the chlorophyll content of the suspension (fig. 4). No final explanations of this relationship is available, but the result shows the importance of experimental chloroplast suspensions of constant chlorophyll content.

C. Finally calcium exchange between chloroplasts and medium in steady-state conditions were also studied.

Therefore isolated chloroplasts were brought in equilibrium with suspensions of different concentrations (see B). Calcium-45, added at that stage, does not affect the steady-state conditions but permits the measurement of the calcium movement between both compartments (comp. 1: incubation medium; comp. 2: chloroplasts) in equilibrium. The transfer of ^{45}Ca from comp. 1 to comp. 2 in the course of time is described by equation 1:

$$Y_t = Y_0 \frac{\lambda_1}{\lambda_1 + \lambda_2} (1 - e^{-(\lambda_1 + \lambda_2)t}) + C \quad (1)$$

in which: Y_t : radioactivity at time t in the chloroplasts

Y_0 : total amount of radioactivity (com) initially added.

$\lambda_1 = \frac{dS_2}{dtS_1}$: transfer coefficient from comp. 1 to comp. 2.

$\lambda_2 = \frac{dS_1}{dtS_2}$: transfer coefficient from comp. 2 to comp. 1.

S_1 : external Ca content

S_2 : internal Ca content

For one particular case, this process is represented, by the curve in fig. 5, which fits very well with the experimental points (first one after 10 sec.). The theoretical intercept with the Y-axis is a measure of the rapid isotopic exchange between ^{45}Ca and ^{40}Ca on the outer membrane. Using the same label in different steady-state conditions, the height of the intercept on the Y axis permits an estimation of the membrane bound calcium (Table 2, column 2).

Since the experiments were done at equilibrium of the system, it is also true that: $\lambda_1 S_1 = \lambda_2 S_2$ (2). In equation (2) λ_1, λ_2 may be calculated from (1); S_1 may be measured.

The calculated S_2 -value in (2) measures the amount of exchangeable Ca in the chloroplasts (Table 2, column 3).

Results in this table give an overall picture of the different forms of calcium-binding in spinach chloroplasts under various concentration steady state conditions.

To a certain extent these data confirm results in table 1.

Table 1 - Ca-efflux from isolated chloroplasts in a Ca-free incubation medium at 20°C over a period of 60 min (results in % of the amount initially taken up, measured by ⁴⁵Ca labelling).

	External Ca concentration: (in µg per ml.)			
	2,35	4,6	22	195
Ca-uptake in µg chlorophyll	0,14	0,41	18	29
Efflux of Ca in % of the initial uptake	80,6%	70,2%	19,2%	8,2%

Table 2 - Relation between the steady state Ca concentration in the incubation medium and different forms of Ca-binding in isolated chloroplasts.

Steady-state Ca-concentration in incubation medium (µg per ml)	Ca-binding in the chloroplasts		
	Membrane-bound Ca (ng per 100 µg chlorophyll)	Exchangeable Ca (ng per 100 µg chlorophyll)	Total Ca (µg per 100 µg chlorophyll)
2,54	22,4	111	1,89
26,2	86	1780	16,6
104	208	1560	21,5
187	240	1880	22,3
270	260	3400	23,2
397	300	2580	23,3

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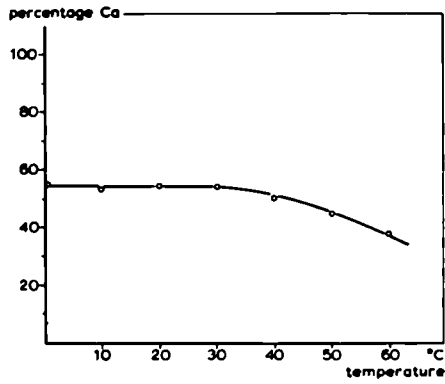


Fig. 1 - Ca content of isolated chloroplasts (% of the total Ca in the suspension) in relation to the temperature.

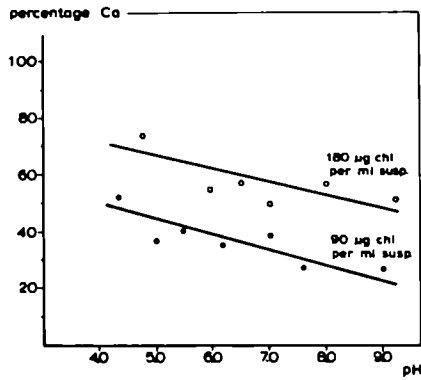


Fig. 2 - Ca content of isolated chloroplasts (% of the total Ca in the suspension) in relation to the pH-value.

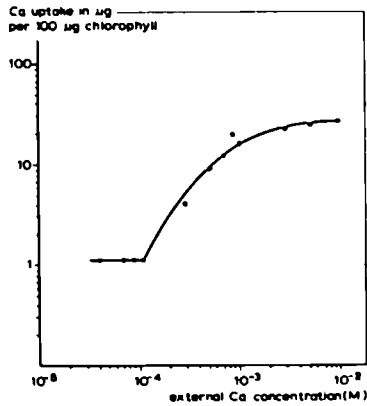


Fig. 3 - Ca uptake, determined by A.A.S.-measurements, in relation to the real external Ca concentration after 60 minutes of incubation at 20°C.

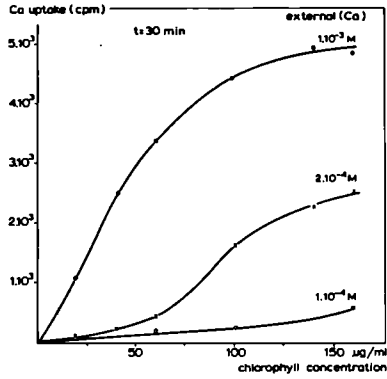


Fig. 4 - Ca uptake by isolated chloroplasts at different external Ca-concentrations in relation to the chlorophyll content of the suspension.

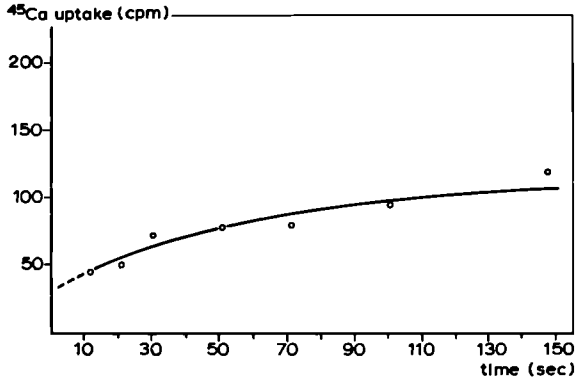


Fig. 5 - ⁴⁵Ca-uptake by isolated chloroplasts under concentration steady state conditions in relation to time.

Resultaten van het project No. 10

Hoofd van het team en wetenschappelijke medewerkers:

C. Broertjes.

Titel van het project: Possibilities of mutation breeding in vegetatively propagated plants, using the adventitious bud technique.

Beschrijving van de resultaten:

The experiments with different plant species, to 1) investigate the number of cells involved in the formation of the apex of adventitious bud, and 2) to demonstrate that commercial results can be obtained within a relatively short period, if the adventitious bud technique is available, are being continued.

In 1971 a number of mutants were produced of the white flowering Streptocarpus ov. Maassens White (a spontaneous mutant of 'Constant Nymph'). A few selected types with reduced growth habit were propagated clonally and compared amongst each other. One will be subjected to a final test in 1973 to decide about its potential commercial value.

Colchicine-induced autotetraploids of the same white cultivar were compared among each other, also on clonal basis. Approx. 20 were selected and propagated for final tests in 1973.

Moreover, crosses were made between some of the tetraploid clones and the diploid white cultivar as well as with a diploid fringed mutant. In addition, several large flowered diploid cultivars with bright pink, red or blue flower colours were used as pollinators. The latter was done in cooperation with Dr. Zeven (IVP) who is interested in the genetics of the (white) mutants. So far no seedlings were obtained, using diploid or tetraploid white as pollinator or as female. It is the experience of Dr. Zeven that white (diploid) as female does produce seeds, but without endosperm; consequently no seedlings are produced.

The work with Streptocarpus is carried out to demonstrate the various possibilities of mutation induction, colchicine-induced polyploidy and cross breeding (or combinations) in a species which can be propagated, amongst others with the adventitious bud technique.

In Achimenes similar work is being carried out. A great number of mutants of the cv. Paul Arnold were judged by a group of specialists, which selected three mutants, one with 3 stars (extremely good) and 2 with one star (promising). The final test will be carried out in 1973. The tetraploid mutants were very disappointing from commercial standpoint of view, they will be discarded except a few, which will be used for demonstration. Additional irradiations and colchicine treatments are being applied to three new pink flowering cultivars to investigate the possibilities in this new group.

Various Kalanchoë mutants (20 of cv. Josine and 10 of cv. Annette) have been propagated to demonstrate the extent of the genetic variability after irradiation, using the adventitious bud technique, as well as to select, in 1973, mutants with commercial value (see also the publication by Broertjes and Leffring).

Of Muscari which very readily produced adventitious bulbils on detached, rooted leaves, several hundreds of adventitious plantlets flowered: approx. 60 mutants were observed, showing variation in size, form and colour of leaves and flowers. So far no chimeras were found, which seem to indicate that bulbils at the base of leaf-parts of this monocotyledonous species also originate from a single cell, like has been demonstrated in various other species. Histological-cytological observations support this idea since they are similar to those of Saintpaulia and other species.

Endymion and Scilla, two other Liliaceae, also reproduce readily from leaf-parts. The adventitious plantlets formed, however, did not yet flower and since so far leaf nor habitus mutants have been observed, at present nothing can be said about the number of cells involved in the adventitious bulbil formation.

The scientific work on adventitious bud formation is waiting until a specialized plant physiologist has been appointed to do this work. The discussions with the 'Adventitious Bud Study Group' are being continued in the meantime.

Publications:

Broertjes, C., and H.Y. Alkema. Mutation Breeding in Flowerbulbs. First Int. Symp. on Flowerbulbs II: 407-411 (1970) (Noordwijk/Lisse).

Broertjes, C. Improvement of vegetatively propagated plants by ionizing radiation. Induced Mutations and Plant Improvement, IAEA, Vienna: 293-299 (1972).

Broertjes, C. Mutation breeding of Achimenes. Euphytica 21: 48-63 (1972).

Broertjes, C. Use in plant breeding of acute, chronic or fractionated doses of X-rays or fast neutrons as demonstrated with leaves of Saintpaulia. Thesis. Agric. Res. Rep. 776; 74 p., 1972.

Broertjes, C. and L. Leffring. Mutation breeding of Kalanchoe. Euphytica 21: 415-424 (1972).

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project - No. 10 bis

Hoofd van het team en wetenschappelijke medewerkers:

S. Roest, G.S. Bokelmann.

Titel van het project: Mutation breeding and radiation induction of self- and cross-compatibility in *Pyrethrum*.

Beschrijving van de resultaten:

1. Adventitious root formation of flowerstem-explants in vitro.

The mineral nutrition proved to be very important. The microelements of Heller did not influence the process. In the presence of the macroelements according to Knop ($\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, KNO_3 , KH_2PO_4 , $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) a considerable stimulation of the adventitious root formation was found. A separate addition of $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$, also yielded a good rooting response. Normally this salt was added to the medium in a concentration of 250 mgr/l. In the concentration range 0, 125, 250, 500 and 1000 mgr/l the best initiation and elongation of a fair number of main roots was observed at the concentrations 500 and 1000 mgr/l $\text{Ca}(\text{NO}_3)_2 \cdot 4\text{H}_2\text{O}$. Of the auxins, indoleacetic acid (IAA), indolebutyric acid (IBA) and naphthaleneacetic acid (NAA), all applied in the concentrations 10^{-7} , 10^{-6} and 10^{-5} g/ml, the optimal concentration for the initiation of the root primordia appeared to be 10^{-5} ; an optimal outgrowth of the root initials, however, was realized at 10^{-7} . The average length of the main roots decreased when the period, during which 10^{-5} auxin was applied, increased. If the explants were incubated continuously on a medium with 10^{-5} auxin, the main roots practically did not elongate and never grew into the medium. On a medium with 10^{-7} auxin or after transfer to a medium without auxin, the adventitious roots strongly elongated and grew into the medium.

2. Root- and shoot formation of shoot cuttings in vivo.

Removing the apical bud of shoot cuttings appeared to be a very important preparation procedure.

By removing the terminal bud the outgrowth of axillary buds was stimulated and also enhancement of the rooting percentage and number of main roots was noticed. The evidence clearly supported the supposition that the growth of lateral buds is checked, directly or indirectly, by endogenous auxins synthesized in the apical buds (apical dominance). The apical dominance of the terminal bud could be restored by the application of exogenous auxins. Auxin solutions of IAA, IBA and NAA in relative high

concentrations and applied during 24 hours, almost completely inhibited the outgrowth of lateral buds; this inhibiting effect was less pronounced when powder formulations of the auxins, in relative high concentrations, were used.

In conformity with the adventitious root formation of flowerstem-explants in vitro, the initiation of a high number of main roots of shoot cuttings in vivo is favoured by relative high auxin concentrations and the outgrowth of the root primordia is stimulated by relative low concentrations of IAA, IBA and NAA.

Adventitious bud formation and the outgrowth of axillary buds in vitro.

In the annual report 1971 the development of shoots from capitulum-explants was announced. In order to improve this process of shoot development, the influence of different factors was considered in detail on a slightly modified basic culture medium of the following composition: 0,6% Difco Bacto-agar, Knop's macro- and Heller's microelements (both at half strength), 2% sucrose and 6-benzylamino purine (BA) at 10^{-6} g/ml. The development of shoots decreased when the age of the capitulum increased, when the capitulum was divided into more than 2 segments and when the disc and ray florets were not or just completely cut off. Without mineral nutrition or after the addition of only the microelements according to Heller, shoots did appear, but their growth was retarded. The development of the shoots was stimulated by the addition of the macroelements according to Knop to the culture medium. Shoots did not develop when sugar was absent in the medium. Of the sucrose concentrations 0, 0,5, 1, 1,5, 2 and 2,5%, the optimum concentration appeared to be 0,5%. In the concentration range 0, 10^{-8} up to 10^{-4} g/ml BA shoot development did not occur in the absence of BA or at BA 10^{-8} g/ml. A period of continuous darkness, during the first weeks of the experiment, enhanced the elongation of the shoots. When the explants were exposed to the temperatures 9, 13, 17, 21 and 25 °C, the most rapid development of the shoots was obtained at 17 °C. Only a few shoots grew out to a length of approximately 1 cm; the majority of the shoots, however, reached a length of only a few millimeters. The development of the shoots was almost completed after an incubation period of 5 weeks. The most elongated shoots were isolated from the capitulum-explant. The basal end of the shoot was treated with 1% IAA (on talc basis) and subsequently the shoot was transferred to unsterilized soil (a mixture

of leaf mould and sand). A high relative humidity was maintained and within 3 weeks adventitious root formation was observed. Consequently, within a period of 2 months, starting incubation in vitro of the capitulum-explants, complete plantlets were produced, which afterwards fully developed to normal plants.

Anatomical observations showed that a majority of the shoots were formed adventitiously and originated from very few or even a single cell of the epidermis of ovaries of disc florets. Irradiation of capitulum-explants, followed by the de novo generation of adventitious buds, might produce solid mutants and chimerism would be avoided or at least greatly reduced. This method possibly offers a contribution to mutation breeding and radiation induction of self- and cross-compatibility in Pyrethrum. On that account recently a number of capitulum-explants were irradiated with different doses of X-rays. In conformity with the result obtained with Pyrethrum, shoot development was also observed, according to the same procedure, from capitulum-explants of 15 other Compositae and from flower-explants of 2 plant species, which belong to other plant families.

4. Ecological research.

In the temperature range 9, 13, 17, 21 and 25 °C flower induction and initiation of flower-heads were only realized at 9 and 13 °C (vernalization). If night-temperatures of 9 and 13 °C were followed by day-temperatures of 17, 21 and 25 °C then flower induction and initiation of flower-heads were greatly nullified (devernalization). Initiation of a fair number of flower-heads occurred, when the cold period (9 °C continuously) lasted for at least 6 weeks. The subsequent development of the flower-heads and the realization of flowering were stimulated by temperatures of 21 and 25 °C.

Publications:

Bokelmann, G.S. and Roest, S.: Spruitvorming bij in vitro gekweekte bloemhoofdjes van Pyrethrum, 8e Weefselkweekdag Baarn (1972).

Bokelmann, G.S.: Vegetatieve vermeerdering van Chrysanthemum cinerariaefolium Vis. in vitro. Verslag ITAL (1972).

Roest, S. and G.S. Bokelmann: Vegetative propagation of Chrysanthemum cinerariaefolium Vis. in vitro. Scientia Horticulturae 1 (1); 120-122 (1973).

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project No. 11.

Hoofd van het team en wetenschappelijke medewerkers:

R.M. Ecochard, M. Matteoli.

Titel van het project: Research on the nature of induced and spontaneous mutations.

Beschrijving van de resultaten:

Induction and nature of unstable chlorophyll mutants in tomato

(*Lycopersicon esculentum* Mill)

a. Further studies on gene instability in tomato mutants C₆, C₁₁ and C₁₂

1. The fifth back-cross generation of the C₆ mutant to Moneymaker was extensively studied with special attention to phenotype/genotype correlation for conversion activity, i.e. probability of Aa → aa. This activity was found to be constant, 0.66, in all heterozygote plants of this generation. In any individual, the proportion of chlorophyll deficiency is entirely determined by random distribution of Aa and aa tissues during ontogenic development. Subsequently the percentage of deficient segregants in the offspring derived from a given fruit only depends on the phenotype of the L_{II} layer at the location of that fruit: when the L_{II} is entirely mutated (if the L_{III} is also mutated the shoot is rarely viable) the progeny is 100% white-lethal. Smaller proportions of lethals reflect an heterogeneity of the parental L_{II} tissues.
2. The C₁₁ gene was spontaneously stabilized after conversion, at an early stage. It has been already back-crossed four times to Moneymaker, so that it is now introduced in an almost pure genetic back-ground.
3. The three mutants were previously crossed with each other, and the F₂ was analysed this year. In each case the segregation found fitted a 9 : 7 model at 0.95 probability level, so confirming independence of the genes. Moreover, in this material also, our interpretation of the mechanism of gene instability is fully substantiated.
4. Localization of the genes: the C₁₁ mutant had been crossed in 1971 with 9 testers, representing 25 different marker genes (15 expressed at the seedling stage) distributed over the whole chromosome map. Two to four thousand F₂ seeds were harvested in 1972 for each cross. Tests have shown that, according to the crossing-over percentage of Butler 17 "marker e" (entire leaves) and Butler 13 "marker d" (divergence), both on chromosome 4, the gene of the C₁₁ mutant is probably also

situated on this chromosome.

Crosses were also initiated for all three mutants with a series of 8 different trisomics (among the 12 existing) cytologically selected. This alternative method is theoretically shorter, but crossings are difficult to perform with that aberrant material. Both approaches are used simultaneously.

5. Attempts to de-repress our mutants with chemicals were continued using 10 different mutagenic substances, which are mainly active on DNA, m-RNA and on chromosome protein. The final results are not yet available.

With C₁₁ so far no complete de-repression with chemicals was found to be heritable. After gamma treatment a family was isolated, which is now in the third generation, and where cotyledons and leaves are markedly spotted with green flecks. This effect might be due to an allele of the C₁₁ gene characterized by a partial de-repression.

b. Induction of new mutants

New pollen irradiations respectively with X-rays, U.V.-rays and thermal neutrons were carried out with the aim of originating new cases of gene instability in the tomato and studying the conditions of their occurrence. Analysis of the progeny is in progress.

Publications:

Annual report 1972 of the Association EURATOM-ITAL.

Ecochard, R. New cases of somatic conversion (paramutation) in tomato (*Lycopersicon esculentum* Mill.)

Theoretical and Applied Genetics 42, 189-195 (1972).

Ecochard, R. and Merckx, G.

A primary monosomic for chromosome 5 in the tomato. Caryologia (in press).

Hoofd van het team en wetenschappelijke medewerkers:

A.J.G. van Gastel, F. Carluccio

Titel van het project: Induction of self-compatibility in dihaploid *Solanum*.

Beschrijving van de resultaten:

A self-incompatible dihaploid clone of *S. tuberosum* L. (derived from the cv Gineke) was exposed during anthesis and seed-set to chronic gamma irradiation at dose-rates ranging from 0.3 to 14.3 rad/hr.

The results obtained (table 1) indicate that a small amount of seeds are produced after self-pollination, in both the control and the irradiated series.

Table 1: Effects of different dose-rates (0.3 to 14.3) of gamma rays on fruit- and seed-set in self-incompatible dihaploid *Solanum tuberosum* L.

Dose rate rad/hr	Number of fruits with seeds	Total number of seeds	Average number of seeds/fruit
Control	2	51	25.5
0.3	-	-	-
0.6	1	22	22
1.0	-	-	-
1.8	-	-	-
2.5	4	251	62.7
4.0	-	-	-
7.0	1	2	2
14.3	-	-	-

All the selfed progenies were test-crossed to determine the cause of the seed-set. The results (table 2) showed that all tested plants in the control series were self-incompatible, whereas self-compatible and self-incompatible plants were obtained in the irradiated populations. After vegetative propagation of self-compatible plants, self-compatible and self-incompatible cuttings were found. This indicates that the self-compatible plants and the selfed seeds probably had a chimaeric structure.

Table 2: Segregation for self-compatibility in the progeny of gamma irradiated plants after self-pollination.

Dose-rate rad/hr	number of M ₂ -plants tested	Response of M ₂ -plants to self-pollination	
		self-incompatible	self-compatible
Control	8	8	-
2.5	23	8	15
7.0	2	2	-

A mutation in the pollen mother cell would not have induced chimaeric plants, therefore it is tempting to conclude that a mutation was induced in the embryo.

The results are summarized in figure 1.

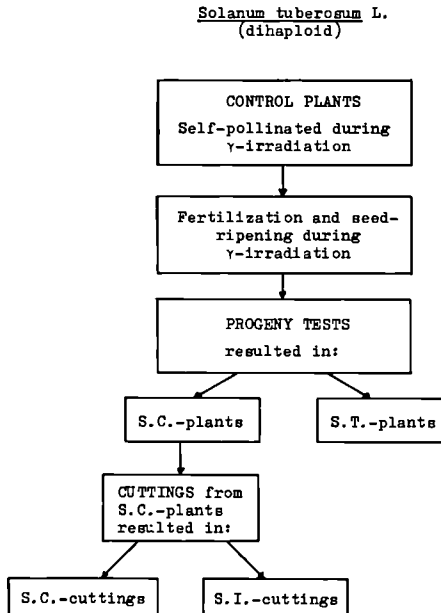


Figure 1: Scheme of results after chronic gamma irradiation of Solanum tuberosum L.

(S.C. = self-compatible; S.I. = self-incompatible)

Publications:

Gastel, A.J.G. van and Carluccio, F. Self-compatibility in the progeny of a dihaploid Solanum tuberosum clone. Incompatibility Newsletter 1, 29-31 (1972).

Annual report 1972 of the Association EURATOM-ITAL.

Hoofd van het team en wetenschappelijke medewerkers:

A.J.G. van Gastel, D. de Nettancourt, G. Merckx.

Titel van het project: Spontaneous and induced mutations at the S-locus: a comparative analysis on the origin and nature of constructive (generation of new alleles) and negative (genetic losses) mutations.

Beschrijving van de resultaten:

A. Mutation spectrum analysis at the S-locus of Nicotiana alata
Link and Otto

The aim of the experiments is the comparison of self-compatibility mutations induced by different mutagenic treatments.

- chronic gamma irradiation

A clonal population derived from a single S_2S_3 individual was exposed, at the flowering stage, to different dose-rates of gamma-rays. Whereas the results obtained clearly indicated that chronic irradiation was very effective for inducing seed-set upon selfing, only few cases of permanent S-mutations could be recorded among the M_2 progenies.

All mutations were of a negative nature (induction of genetic losses or showing competition effects leading to self-compatibility) and not a single constructive change (formation of a new S-specificity) could be recorded. Comparing these results with those obtained after acute exposure to X-rays, chronic irradiation appears to be far less effective than acute treatment for inducing self-compatibility mutations.

Moreover both, chronic and acute irradiations seem to be unable to generate new self-incompatibility alleles.

- acute irradiation with X-rays and fast neutrons

All the self-compatibility mutations obtained after X-irradiation of pollen mother cells (P.M.C.) have been tested. It seems that X-ray treatment can be successfully used for the induction of negative mutations. The test-crosses revealed that in all cases pollen-part mutations of the S-locus were obtained. The majority of the mutants obtained after P.M.C. irradiation with fast neutrons also are of pollen-part nature.

- treatment with Ethyl methanesulfonate (EMS)

In the Department of Genetics, University of Wageningen (Prof. J.H. van der Veen) seeds and P.M.C.'s have been treated with methanesulfonate (EMS) by M.A. Beek, L. van Soest and J. Simonsma.

The results obtained indicate that seed treatment is not effective for the induction of self-compatibility mutations. Large amounts of selfed seeds from this strictly self-incompatible species were obtained.

After treatment of P.M.C.'s with EMS several mutant seeds were obtained. A progeny test will reveal if this seed-set is due to permanent (stable S-mutations) or to non-permanent (physiological effects or revertible mutations) changes.

B. The induction of constructive mutations at the S-locus of Nicotiana alata Link and Otto

The aim of the experiments is the determination of the natural and experimental conditions which lead to the formation of constructive mutations at the S-locus (generation of new S-alleles coding a new incompatibility protein).

Up to now X-ray, EMS and U.V. treatment of excised leaves have been used for the induction of new specificities. Not a single case of a new specificity was found. This may be due to the small number of plants used and, in the case of the U.V. irradiation to absorption of the major part of the irradiation by the cuticula.

C. The analysis of the factors and mechanisms involved in the generation of new S-alleles (test-species: Lycopersicon peruvianum Mill)

This part of project 13 is mainly carried out in Rome (D. de Nettancourt).

- The first two steps, necessary for testing the Edström theory that constructive mutations are due to the reactivation of allelic copies previously stored during outbreeding are finished (Rome). In Wageningen a repetition of this experiment with S₆ and S₇ alleles is in progress: the first step (selection of S-genotypes via a diallel cross) is finished.

- Reversion tests, to find out if the new S-allele can revert to the original allele, are finished. In the last series not a single case of reversion has been obtained.

Publications:

Gastel, A.J.G. van. Spontaneous stylar-part mutations at the S-locus in Nicotiana glata Link and Otto.

Incompatibility Newsletter 1. 12-13 (1972).

Gastel, A.J.G. van and Nettancourt, D. de. The evaluation of gametophytic self-incompatibility as a test-system for estimating the relative efficiency of different mutagens. I. The effects of relatively low dose-rates of chronic gamma irradiation on Nicotiana glata Link and Otto (in preparation).

Annual report 1972 of the Association EURATOM-ITAL.

Hoofd van het team en wetenschappelijke medewerkers:

A.J.G. van Gastel, D. de Nettancourt.

Titel van het project: Establishment of linkage relationships with the S-locus of self-incompatible plants and identification of the S-bearing chromosomes.

Beschrijving van de resultaten:

A. Establishment of linkage relationships in *Nicotiana alata*

Link and Otto

The aim is to induce in *Nicotiana alata* Link and Otto marker mutations on a large scale. The induced mutations will be tested for linkage relationships with the S-locus. This approach may permit early identification of S-genotypes. Up to now excised leaves have been treated with X-rays and methanesulfonate (EMS). Three morphological mutants have been recorded among the plants derived from the treated leaves. Several tests were carried out to establish the genetic nature of these mutations. Not a single case of transmissibility of these mutations was found.

These results may be explained in one of the following ways.

- association of the marker-mutation with a factor conferring pollen sterility or embryo-abortion.
- presence of the mutation restricted to the L₁ and L₃ layer of the mutants.
- non-genetic nature of the mutation (physiological disturbances restricted to the treated generation).

B. Identification of the S-bearing chromosome in *Nicotiana alata*

Link and Otto

Via trisomic *Nicotiana alata* Link and Otto plants we try to localize the S-locus. Trisomic plants can be obtained via back-crosses of triploid plants with the original diploid clone.

According to the competition theory, the plants trisomic for the S-bearing chromosome are self-compatible.

At the moment the progeny of the cross tetraploid-diploid *Nicotiana alata* is analysed. The first results indicate that the triploid plants are in most cases incompatible with all related genotypes. We do not yet have an explanation for these results; one should at least expect a positive result in some crosses with S-homozygotes.

Publications

- Carluccio, F., Gastel, A.J.G. van and Nettancourt, D. de.
Modification of chromosome 3 in a leaf-propagated S.S. clone of Nicotiana alata. Incompatibility Newsletter 1. 6-7. ²(1972).
- Carluccio, F., Nettancourt, D. de and Gastel, A.J.G. van. On a possible involvement of chromosome 3 in the formation of self-compatibility mutations in Nicotiana alata Link and Otto. Eucarpia-IAEA-FAO conf. Mutations and Polyploidy, Bari, 1972, in press (1973).
- Gastel, A.J.G. van, Dijkhuis, P. and Nettancourt, D. de. The inheritance of marker mutations obtained by means of the leaf-propagation technique in Nicotiana alata. Incompatibility Newsletter 1. 14 (1972).
- Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project No. 15

Hoofd van het team en wetenschappelijke medewerkers:

G. Bredemeijer.

Titel van het project: Biochemical aspects of self-incompatibility in Lycopersicum peruvianum Mill. and Nicotiana alata Link and Otto.

Beschrijving van de resultaten:

1. Identification of S-genotypes in leaves and callus of Nicotiana alata Link and Otto and L. peruvianum Mill.

Some preliminary experiments carried out in 1971 indicated a possible relation between the S-genotype and the peroxidase isoenzyme pattern of a leaf.

A more detailed study, considering the influence of the developmental stage and the influence of the genetical background was carried out in 1972. It was found that the genetical background and the developmental stage influence the peroxidase isoenzyme composition of the leaf. Consequently, it was difficult to establish the presence of specific S-peroxidases in the leaf. Moreover, it was unknown whether the S-gene is active in vegetative parts of the plant.

These problems made it necessary to switch over to determination of S-peroxidases in the styles and to see whether the same peroxidases occur in leaves.

In cooperation with M. Giacomelli and M. Devreux (C.N.E.N., Casaccia, Italy) a study on the possible relation between S-genotype and peroxidase isoenzyme pattern, using tobacco callus was started. In this way the influence of the developmental stage and of the presence of pigments in the leaves is avoided.

2. Identification of S-peroxidases in tobacco styles

In the first place the influence of the developmental stage of the flower was investigated. It appeared that the peroxidase isoenzyme pattern of the style changed both qualitatively and quantitatively during floral development and senescence. So, in further experiments styles at the same growth stage (anthesis) were used. A second experiment, on the influence of the genetical background, was initiated.

3. The involvement of peroxidase isoenzymes in the self-incompatibility reaction.

Changes in peroxidase isoenzyme patterns of un-, self-, and cross-pollinated tobacco styles were compared to investigate the possible role of peroxidases in the determination of self-incompatibility. Some preliminary experiments showed that the activity of several peroxidase isoenzymes increase during both compatible and incompatible pollen tube growth. These changes are much more pronounced after cross-pollination than after self-pollination. In some experiments qualitative differences in the peroxidase isoenzyme patterns of self- and cross-pollinated styles were observed.

Publications

1. Bredemeijer, G.M.M. (1972). Identification of S-genotypes in leaves of Lycopersicon peruvianum Mill and Nicotiana glauca Link and Otto. Incompatibility Newsletter 1. 28-29.
2. Bredemeijer, G.M.M. (1973). Peroxidase activities and peroxidase isoenzyme patterns during growth and senescence of the unpollinated style and corolla of tobacco plants. Acta Bot. Neerl. 22. (in press).
3. Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project - No. 16.

Hoofd van het team en wetenschappelijke medewerkers:

H.M.G. Groot.

Titel van het project: The radiation dose-fractionation effect in Saintpaulia.

Beschrijving van de resultaten:

The objective of this project is to identify the origin of the radiation dose-fractionation effect, which has been observed by C. Broertjes in *Saintpaulia ionantha* H. Wendl cv Utrecht.

Leaves, submitted to a low initial irradiation dose, are able to stand a second much higher dose than the controls. The optimum time interval between both irradiation treatments is 8 to 12 hours. This effect has been observed with X-rays as well as with fast neutrons. In the present study so far exclusively X-rays have been applied. A few possibilities about the origin of the dose-fractionation effect were considered, such as changes in the metabolic activity of the leaves, the transport of a radiation-induced protective agent, etc. Respiration of the leaves, which expresses to a certain extent the metabolic activity, has been determined at room-temperature in cooperation with Dr. G.R.M. Verfaillie using the Infra Red Gas Analyser. The intensity of the CO₂-production decreased with time, but was in the irradiated leaves (500 rad), as compared to the not-irradiated leaves, somewhat higher during the first 24 hours.

Since environmental factors have great influence on metabolic activity, the effect of temperature, oxygen, nitrogen, etc. was and will be further investigated. Different temperature treatments (6°C, 13°C, 20°C and 29°C) have been applied to detached leaves in sealed polyethylene bags, 24 hours before the first irradiation, between irradiations, and 24 hrs after the second irradiation. In addition, similar treatments were applied exclusively during the time interval between the two irradiations. So far, no results can be presented. Dose-fractionation experiments have been carried out with leaves, sealed in polyethylene bags. The gas-environment inside such bags has been investigated, using the gaschromatograph (in cooperation with ir. G. Sauer). Both with irradiated (500 rad) and not-irradiated leaves the CO₂-concentration increased with time, reaching a maximum after 3 to 5 days (2,5 vol % CO₂), whereas the O₂-concentration decreased to 17 vol % O₂ in the same period. Since, after 8 - 12 hrs, the CO₂-concentration was approx. 1% and the O₂-concentration approx. 20%, these minor changes in

the gas-environment inside the polyethylene bags are probably not a major factor in the development of the dose-fractionation effect. The situation is completely different when gastight bags (Straboplex FE 1550) are used. In such bags the CO₂-concentration steadily rose up to 17% after 11 days, whereas the O₂-concentration decreased to a value of approx. 2%, again using both types of leaves. Leaves, stored in these bags for periods longer than 11 to 14 days, died.

In order to investigate whether a radiation-induced protective agent is responsible for the dose-fractionation effect, petioles were irradiated with high X-ray doses, with or without pretreatment of either the leaf blade or the petiole. In a preliminary experiment three initial doses (500, 1500 and 3000 rad) as well as two time intervals (8 and 24 hrs) were applied. Results are not yet available.

Finally it has been demonstrated that the variance, using "at random planting" of leaves from different treatments in one box is essentially the same as with the previously used method (leaves of a given treatment in the same box). Since "at random planting" has additional practical advantages, this method was chosen for the future experiments.

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project -No. 16 bis
Hoofd van het team en wetenschappelijke medewerkers:
R. Ecochard

Titel van het project: Investigations on thermal neutron sensitivity of living organisms.

Beschrijving van de resultaten:

The effect of thermal neutron irradiations on Lemna minor L. was further studied.

1. Study of growth rate under chronic exposure

As in previous studies Lemna populations were grown on solutions, containing either ^{14}N or ^{15}N and either ^{10}B or ^{11}B , and chronically irradiated in the reactor (thermal neutron flux: $2.5 \cdot 10^7 \text{ n cm}^{-2} \text{ sec}^{-1}$ with 5 rad/h gamma contamination). The growth curves were scored, either throughout the exposure, or after transplanting treated samples onto control non-irradiated solutions.

All curves fitted a sigmoidal model

$$\frac{dN}{dt} = aN - bN^2$$

where a (initial growth potential) reflects the previous history of the inoculum, and b (saturation factor) integrates the limiting growth conditions during development. The results were:

- 1 - In chronically exposed populations, both nitrogen and boron substitutions affect significantly b, but in contrast to earlier experiments with tomato pollen boron is prevalent (1.55 and 2.57 ratio in two independent experiments),
- 2 - A similar response is manifested in the a parameter in the post-irradiation experiment.

As for b this experiment shows, besides a greater response to both treatments and a prevalence of the nitrogen effect, that,

- 3 - The damages caused by the N,p reaction (^{14}N), although moderate at the onset, irretrievably impair the subsequent growth in unexposed conditions,
- 4 - The damages due to the N,α (^{10}B) reaction, initially more severe, are progressively repaired.

The above results bring out interesting complements of information. Greater damages with boron are consistent with the larger B/N ratio found in fresh Lemna tissues, as compared with other plant material, especially pollen (table 1). The nature of injuries also appear different: these due to the N,p reaction, localised in or near the cell nucleus are mainly chromosomal, and therefore non-repairable as well as self-replicating. In contrast, injuries to the cytoplasm or cell membrane sites where boron is involved are most probably physiological in nature and concern cell components or structures with a fast turn-over.

2. Biochemical effects

The investigation was conceived by Prof. M. Theller and conducted partly in the Association's Institute (treatments) and partly in Rouen University (analysis).

Lemna bulk populations grown on ^{10}B and ^{11}B in the presence of pure ^{15}N were submitted to 24 h. thermal neutron irradiation, given ^{14}C -sodium bicarbonate during exposure, and fixed in boiling alcohol immediately after the irradiation treatment. The material was then submitted to separation and dosage of cellulose, pectic substances, lipids, sugars and aminoacids, and the activity measured by autoradiography of each treatment.

It was found that, in ^{11}B material, the cellulose activity was twice as high as in ^{10}B material which, in turn, contained two active sugars and one active amino-acid (serin) in excess.

Another experiment was then done in summer 1972, this time on 3 treatments, $^{10}B^{15}N$, $^{11}B^{15}N$ and $^{11}B^{14}N$, with more material and a higher total activity, in order to repeat and refine the analysis, and especially to compare the ^{10}B and ^{14}N effects, on the carbone metabolism.

Working out the complete results is in progress (autoradiography of the chromatograms needs a long contact). However, the first results mentioned above are in favour of a boron localization and action at the level of the cellulose membranes.

Besides the work on Lemna minor L., the possibility of nucleus inactivation at the plant fertilization stage was also considered.

Here the idea was to pollinate a ^{15}N plant with ^{14}N pollen,

or reciprocally, and to expose the flowers to thermal neutrons. There is no DNA synthesis until zygote formation, the 'hot' gametic nucleus would possibly be selectively destroyed and, hence, a haploid be produced, either parthenogenetic or androgenetic.

The advantages of being able to rise haploid plants on request (cf. Devreux) and the a-priori feasibility of the experiment urged us to try it, with tomato.

Results will be known in the beginning of 1973.

Table 1: Chemical composition of different plant parts expressed in % of the fresh tissues.

% fresh weight	<u>Vicia faba</u> Meiotic buds	<u>Lycopersicon</u> <u>esculentum</u> Ripe pollen	<u>Lemna minor</u> Total body
H ₂ O	82.30	2.78	95.80
C	7.94	46.07	1.70
O	78.51	32.07	86.60
N	1.37	9.28	0.29
H	10.26	7.31	10.92
B	0.00262	0.00266	00.000617
ash	1.92	5.27	0.49
total	100.00	100.00	100.00

Publications:

Theulier, M. and R. Ecochard, Rôle physiologique du bore révélé par la réaction de Lemna minor aux neutrons thermiques. I. Effet de l'irradiation sur le métabolisme ¹⁴C (in press).

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project AA 009 - No. 18.

Hoofd van het team en wetenschappelijke medewerkers:

C. Broertjes.

Titel van het project: Organization and coordination of applied mutation breeding.

Beschrijving van de resultaten:

During 1972 again many scientists and commercial plant breeders in the Netherlands, in other common market countries but also from various other places in the world, used the service of the Association (general information about the possibilities of mutation breeding in crops of interest; requests for literature on a given subject, irradiation of material as well as advice and support in general for running projects). Six new irradiation projects were started in 1972, all but one with asexually propagated ornamentals (Alstroemeria, Azalea, Lilium and Tulip (2)) and one with a seed propagated ornamental Lilium species. The total number of mutation projects, started since 1959, now amounts to 179, namely cross-pollinators 17, self-pollinators 23 and vegetatively propagated species (including apomicts) 139. A number of these projects have been discontinued for various reasons, so that the number of "active" projects is estimated at approx. 100.

In 1972 one Alstroemeria mutant was commercialized under the name of "White Wings" (an almost white mutant of the yellow flowering cv. Orchid Flower) by the commercial breeder M.C. van Staaveren, Aalsmeer, the Netherlands. A chrysanthemum mutant, an improved type of Indianapolis yellow (darker yellow, better growth, better flower form) was reported to be commercialized under the same name in 1970.

Promising results were reported in various crops and it is expected that mutant varieties will be released in the near future of Achimenes, Alstroemeria, Chrysanthemum, Kalanchoë, Lilium, onion, Poa, Streptocarpus and various bulb crops.

Publications:

Broertjes, C. and H.Y. Alkema. Mutation breeding in Flowerbulbs. First Int. Symp. on Flowerbulbs II: 407-411 (1970) (Noordwijk/Lisse).

Broertjes, C. Improvement of vegetatively propagated plants by ionizing radiation. Induced Mutations and Plant Improvement, IAEA, Vienna: 293-299 (1972).

Broertjes, C. Mutation breeding of Achimenes. Euphytica 21: 48-63 (1972).

Broertjes, C. Dose-fractionation studies and radiation-induced protection phenomena in African Violet. Survival of Food Crops and Livestock in the Event of Nuclear War, BNL, Upton, L.I., N.York: 325-342 (1971).

Broertjes, C. Use in plant breeding of acute, chronic or fractionated doses of X-rays or fast neutrons as demonstrated with leaves of *Saintpaulia*. Thesis. Agric. Res. Rep. 776; 74 p., 1972.

Broertjes, C., and L. Leffring. Mutation breeding of *Kalanchoë*. *Euphytica* 21: 415-424 (1972).

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project No. 19

Hoofd van het team en wetenschappelijke medewerkers:

S.C.E. Romkes, J.H. Becking.

Titel van het project: Influence of irradiated medium on micro-organisms, (Sub-title: Uptake and localization of cytotoxic compounds induced by irradiation of food).

Beschrijving van de resultaten:

The research on the inhibition of growth in micro-organisms was continued. Since the very sensitive strain Micrococcus morshuae was not available, a screening programme for strains sensitive to irradiated medium was set up.

Salmonella cameronensis skin was homogenized with a ultra turrax mixer and the homogenate plated out in several dilutions on plate-count agar with 0 and 3 % NaCl and on nutrient agar at 0 and 3 % NaCl.

More than two hundred and fifty strains were isolated.

These were again plated on the same medium, irradiated at 0, 100, 200, 300 and 500 krad.

This programme yielded about 27 strains of varying sensitivities. Of these, five strains could be labelled very sensitive. These strains have not been classified as yet.

NaCl in the medium at the level indicated, can afford some protection against irradiated medium to a number of strains, while it seems to make other strains more sensitive.

It proved possible to adapt the sensitive strains to irradiated medium, by repeated passages through medium, irradiated with 100 or 200 krad of ⁶⁰Co gamma rays.

Sensitive strains and their counterparts are being compared in transport and metabolism studies.

As references, strains and mutants with known metabolic pathways were selected from a culture collection in order to acquire a spectrum of physiological types.

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project - No. 20.

Hoofd van het team en wetenschappelijke medewerkers:

H. Stegeman

Titel van het project: The radiation and heat resistance of bacterial spores.

Beschrijving van de resultaten:

As reported in 1971 alterations in the exchangeable fraction of the metal content of spores by acid treatment influenced considerably the sensitizing effect of pre-irradiation to a subsequent heat treatment. This year the investigation was concerned with alterations in metal content of spores affected by the cultural conditions during sporulation and their influence on the resistance to radiation and combined radiation/heat treatments.

· Effect of manganese in the sporulation medium

To obtain spores of *Bacillus subtilis* ATCC 6633 with different manganese contents the manganese concentration in the sporulation medium (trypticase soy broth) was varied. The addition of manganese up to a level of one ppm increased the spore yield and heat resistance of the produced spores, but the radiation resistance and the sensitizing effect of pre-irradiation to a subsequent heat treatment was unchanged (Table 1). The addition of manganese (0.1 - 10 ppm) to nutrient broth fortified with 0.4 percent of yeast stimulated sporulation of *Bacillus stearothermophilus* NCA 1518 smooth variant; the thermal resistance of the spores increased slightly and the radiation sensitivity was unaffected.

Spores of *Bacillus stearothermophilus* NCA 1518 rough variant, produced in a chemically defined liquid sporulation medium with different manganese concentrations, varied in heat sensitivity, but there was no difference in radiation resistance.

In all the above experiments the lytic enzyme system, which autolyses the vegetative and mother cells, was mainly inhibited at low levels of manganese in the growing media. Therefore it was difficult to clean spore suspensions from vegetative cells and debris.

· Effect of calcium in the sporulation medium.

The addition of 136 ppm calcium to the growing medium (trypticase soy broth, enriched with one ppm manganese but without dipotassium phosphate) gave spores of *Bacillus subtilis* ATCC 6633 with an increased heat

resistance; the radiation resistance and spore yield was slightly decreased and the loss of heat resistance after-pre-irradiation was unchanged (table 2).

Higher levels of calcium in the sporulation medium resulted in metal precipitates and in abnormal spores.

These results are in agreement with the idea that the mechanisms responsible for the thermo-and radiation-resistance of spores are not correlated. The specific roles of the divalent cations, manganese and calcium, in sporulation and in regulation of heat resistance during sporulation can not directly be associated with the mechanisms responsible for the radiation resistance and for the loss of heat resistance of spores, surviving exposure to low levels of irradiation. Alterations in the metal content of spores by changes in the divalent metallic ion concentration in the sporulation medium can at this moment not be related with the measured spore resistances because data concerning the metal content of the different spore crops, obtained by neutron activation analysis (cooperation with P. Poelstra and N.v.d.Klugt) are not yet available.

Further studies are directed towards the identification of the exchangeable fraction of metal ions responsible for the increased synergistic effect of irradiation and heat.

This work was done in collaboration with Prof.Dr. W. Pilnik (Department of Food Science, Agric. Univ. Wageningen) and Prof.Dr. A.A. Mossel (Department of Food Preservation, Cath. Univ. Louvain).

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Table 1 - Effect of manganese added to the sporulation medium on the spore crop and the resistance to heat and irradiation of *Bacillus subtilis* spores

concentration of manganese in ppm	spore yield (spores/ml)	D _{85°C} -value (decimal reduction time) of heated spores in water	D ₁₀ -value (decimal reduction dose) of irradiated* spores in water	Loss of heat resistance after pre-irradiation* with 100 krad
0	2.10 ²	-	-	-
0.01	2.10 ⁴	-	-	-
0.05	1.10 ⁸	4.3 min	0.16 Mrad	40 %
1.00	1.10 ⁹	12.6 min	0.16 Mrad	40 %

*X-rays.

Table 2 - Effect of calcium added to the sporulation medium on the spore crop and the resistance to heat and irradiation of *Bacillus subtilis* spores

concentration of calcium in ppm	spore yield (spores/ml)	D _{85°C} -value (decimal reduction time) of heated spores in water	D ₁₀ -value (decimal reduction dose) of irradiated* spores in water	Loss of heat resistance after pre-irradiation* with 100 krad
0	1.10 ⁹	6.3 min	0.15 Mrad	40 %
136	2.10 ⁸	13.8 min	0.13 Mrad	40 %

* γ-rays.

Resultaten van het project No. 21

Hoofd van het team en wetenschappelijke medewerkers:

J.G. van Kooy, M.F.J. Bruurs.

Titel van het project: The influence of environmental factors on the radiation resistance of micro-organisms.

Beschrijving van de resultaten:

The experimental work was based on the following observations, which were obtained from the results on the irradiation of fresh meat and on the irradiation-decontamination of fishmeal.

1. The surviving flora of low dose treated fresh meat showed a delay in its development.
2. The residual flora of Enterobacteriaceae in fishmeal and in pellets made from it, although low in number, possessed a very high radiation-resistance.

In case of the growth retardation of microorganisms after irradiation, it seems interesting to study the cause of that delay. An increase of that delay in development of spoilage causing bacteria can certainly contribute to a longer shelflife.

A principle question of this study is: Do irradiated media retard or inhibit the growth of microorganisms ?

The experiments were carried out with Microbacterium thermosphactum, isolated from 60 krad irradiated minced meat. The following media were irradiated: Peptone-NaCl solution, peptone/NaCl/glucose-solution, Peptone/glucose/phosphate buffer-solution, of which either the peptone or the glucose was irradiated.

Although the preliminary results were not always consistent, the following tendencies of the effect of irradiated media on the behaviour of the considered microorganism can be reported:

Inoculation of the M. thermosphactum in 50 krad irradiated peptone NaCl-solution resulted in a considerable reduction of viable cells during the first few days, followed by a slow 'repair'.

Inoculation of the test organism in peptone/NaCl solution 24 hrs after a 50 krad irradiation still caused a reduction of the inoculum.

When the medium is enriched with glucose, the growth rate of the species considered was not affected by a 50 krad dose; but when the latter medium was irradiated with increasing doses in the range from

0.1 up to 2.0 Mrad retardation of growth gradually increased and an almost complete inhibition finally occurred. A heat sterilization treatment of the same medium showed an effect comparable with an irradiation dose of 0.5 Mrad. In case of the phosphate-buffered solution of peptone and glucose an 0.5 Mrad dose did not show an effect on the growth of M. thermosphactum. Clear inhibition effects occurred with 1.0 and 2.0 Mrad. Furthermore, it was found that 1.0 Mrad irradiated peptone has a larger influence on the growth-retardation than glucose irradiated with the same dose. In the experiments with 2.0 Mrad no difference was found in growth-retardation as a consequence of either a glucose or a peptone irradiation.

The results of these preliminary experiments indicate a positive answer to the question mentioned above. Presented data, however, cannot explain the observed effect on microorganisms in low dose irradiated meat.

Experiments concerning the radiation-resistance of Enterobacteriaceae in fishmeal and pellets, were carried out with an Enterobacter, isolated from 600 krad irradiated fishmeal, and this species was cultured in nutrient broth.

Aliquots of fishmeal were infected with the Enterobacter, harvested from nutrient broth, and kept over various saturated salt-solutions in order to maintain, during the experimental period, moisture-contents with desired values.

The following results were obtained:

The viability of Enterobacter in such fishmeal-preparations decreased rapidly when moisture content was between 15 and 25 %. In one experiment 90 % of the initial count was inactivated during the first 8 days; in an other experiment an initial count of $10^8/g$ was completely eliminated after 25 days storage at 25°C over a saturated KNO_3 -solution.

The D_{10} -value of the surviving Enterobacter on successive days during storage was determined. The experimental conditions were not always comparable between different experiments, which may have contributed to rather great variations in the increase of D_{10} -values. One day old fishmeal preparations showed a D_{10} -value of an average of 40 krad; 8 days old samples 150 krad, and after a 3 or 4 weeks storage, the D_{10} -values increased to 260 krad.

Water, but especially the extent to which it is available, seems to play an important role in the radiation resistance of the bacterial cell. The parameter 'Water-activity' (a_w) was chosen as a suitable characteristic for the availability of water for the cell. Subjection of microorganisms to an environment of low a_w is considered as a stress condition for such organisms. Therefore the effect of a_w on the radiation resistance of Enterobacter was studied by means of solutions of various concentrations of glycerol and enriched with nutrient broth. A_w values were 0.99, 0.90, and 0.55 resp. The solutions were inoculated with the Enterobacter-species also used in aforementioned experiments. On successive days after inoculation subsamples of these media were irradiated with doses varying from 0 to 400 krad. The results can be summarized as follows:

The Enterobacter species is inactivated in a medium with $a_w = 0.55$: 40 % of initial count within 24 hrs. Culturing of this organism in media with a_w of 0.90 and 0.99 resp. has little effect on the viability.

D_{10} -value of this species in a medium with $a_w = 0.99$ has an average of 65 krad; the radiation resistance of this species was not clearly affected by ageing of the culture. The D_{10} -value of the Enterobacter in media of $a_w = 0.90$ and 0.55 was 160 and 270 krad resp. Due to the fact that the Enterobacter species died rapidly in a medium of $a_w = 0.55$, determination of dose-survival curves after an ageing period of 13 days was not possible.

The increase of the D_{10} -value takes place in a medium with $a_w = 0.55$ within 24 hrs. Available data cannot explain this fast increase; it should also be realized that the increase of the D_{10} -value is most probably only partly due to a reduced a_w , because a protective effect on bacteria has to be ascribed to the presence of glycerol in this medium.

The data reported here have given rise to the assumption that environmental factors considerably influence the radiation resistance of microorganisms in irradiated foodstuffs. This influence will be the object of further research.

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project

No. 24

Hoofd van het team en wetenschappelijke medewerkers:

J.G. van Kooy, H.G. Heins, D.I. Langerak.

Titel van het project: Coordination of food irradiation research

Beschrijving van de resultaten:

Due to the absence of the project leader (see below) and the reorganization of the Pilot Plant for Food Irradiation the number of activities have been reduced in 1972 and the planning of new irradiation projects was delayed.

Shelf life extension of prepacked peeled and boiled fresh mussels

In cooperation with the Institute for Fishery Products TNO, the influence of gamma radiation on fresh peeled and boiled mussels was investigated.

Doses of 75 and 150 krad gave total shelf lives of 13 and 16 days respectively, when stored at a temperature of 2 degrees Celsius. The organoleptic quality was found to be most acceptable. Not-treated samples were rejected after 6 days.

Sprout inhibition of onions by irradiation

In cooperation with the Sprenger Institute and the 'SNUIF', i.e. the foundation of the Netherlands Onion Federation, earlier investigations were continued. The radiation effect was studied at different stages during dormancy. The best results sofar were obtained with a dose of 6 krad, applied as soon as possible after harvesting and drying. Most bulbs, however, showed internal discolouring after irradiation and storing, namely more or less browning of the vegetation point. Next studies will be focussed on this phenomenon.

Consumer test with irradiated and chemically treated potatoes

In order to increase our knowledge regarding the consumers attitude towards irradiated potatoes, a consumer test was started again, among the personnel of ITAL and of the National Institute for Public Health. The results will be given in the 1973-report.

Intermediate Moisture Foods

The Central Institute for Nutrition and Food Research has carried

out a first trial with prepacked dried meat. Gamma radiation up to a dose of 250 krad realized a keepability of 12 days, at room temperature and 7.5 % moisture content. As this application might be important under tropical circumstances, continuation of these experiments is considered.

Ir. J.G. van Kooij was appointed in 1972 on behalf of the IAEA, Vienna, to assist the staff of the Atomic Energy Commission of Chile, in the development of a food irradiation programme.

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Resultaten van het project - No. 25.

Hoofd van het team en wetenschappelijke medewerkers:

J.G. van Kooij.

Titel van het project: Coordination of research in the field of wholesomeness of irradiated food.

Beschrijving van de resultaten:

1. The total diet feeding test with mini-pigs is terminated.

This two years lasting experiment was very successful in all aspects.

The final histopathological data, however, are not yet available.

From the preliminary results so far obtained, it was concluded, that the feeding of an irradiated diet, consisting of normal human food, during a period of two years has no detrimental effects.

The final results, available in July 1973, will be given in the annual report of that year.

2. The total diet feeding test with domestic pigs of the breed Pietren had to be terminated in July 1972, because of the fact that 20% of the total number of animals died during the first year of this experiment. The health condition of the remaining animals, although carefully selected at the start, was considered as too labile, and a continuation of the test with this breed was no longer justified.

In the meantime, this feeding test was started again with a group of young animals belonging to the much stronger breed "Groot Yorkshire". A few minor changes have been introduced in the testscheme of the new experiment, which may contribute to a prevention of calamities as experienced before.

Publication :

Annual report 1972 of the Association EURATOM-ITAL,

Resultaten van het project - No. 26.

Hoofd van het team en wetenschappelijke medewerkers:

D.Is. Langerak.

Titel van het project: Preservation of fruits and vegetables by means of ionizing radiation.

Beschrijving van de resultaten:

In 1972 a number of experiments have been carried out with peeled potatoes, fresh mushrooms, prepacked onions and endive.

Peeled potatoes.

Previous research in 1971 showed that the keeping quality of peeled potatoes treated with $\text{Na}_2\text{S}_2\text{O}_5$ (sulphite) was considerably improved by irradiation (synergistic effect). However, a disadvantage of this treatment was that the taste was affected at a dipping concentration $> 0,5\%$ and an irradiation dose > 50 krad. In connection with this research the following aspects were investigated.

1. The influence of a dipping concentration of $0,1 - 0,5\%$ $\text{Na}_2\text{S}_2\text{O}_5$ in combination with an irradiation dose of $0 - 110$ krad on quality, shelf life and taste.
2. The influence of irradiation on the absorbed sulphite content in the peeled potatoes. The synergistic effect can indeed be explained by a better absorption of the sulphite in the irradiated potatoes. The sulphite content in the potatoes was measured according to the method Zonneveld & Meyer.
3. Improvement of the available bacteriological sampling method.

The blending method was not satisfactory for peeled potatoes. Therefore the blending of the samples was replaced by the "washing and shaking" technique. Both treatments were statistically compared.

Besides the influence of various washing times and pepton dilutions on the results was studied.

Results

ad 1. With respect to the non-irradiated product, this experiment showed that even a low sulphite concentration in combination with irradiation gave a considerable improvement.

However, the keeping quality decreased at a lower concentration and irradiation dose. Therefore a sulphite concentration of $0,1\%$ combined

with an irradiation dose of 50 krad was not sufficient.

Concerning the taste, the best results were found at the lowest concentration and irradiation dose.

Further research is still necessary for the determination of the optimal dipping concentration and irradiation dose.

ad 2. These measurements show that, immediately after treatment, the sulphite contents decrease at an increasing irradiation dose. According to the t-test, the difference between the non-irradiated and irradiated samples were significant.

The synergistic effect of the sulphite treatment and irradiation therefore cannot be explained by a better sulphite absorption.

ad 3. The t-test showed that the difference between both treatments were not significant (calculated $t = 1.04$, tabulated $t = 2.18$ at $P = 0.05$). Without any objection the blending method can be replaced by the washing method.

The study using various washing times and dilutions showed that a dilution of 1 : 1 and a washing time of 10' was sufficient.

Fresh mushrooms.

Two experiments have been carried out. The aim of the first experiment was to study the possibility to store irradiated fresh mushrooms for the canning industry without quality deterioration. This method offers the canning industry the possibility to purchase fresh mushrooms in a period of low prices.

This research showed that, with regard to the keeping quality of fresh mushrooms for preservation, a gamma irradiation of 200 krad gives the best results.

The second experiment was carried out in collaboration with the Sprenger Institute and the Mushroom Experimental Station in Horst. The aim of this research was to study the influence of irradiation on the keeping quality of the new variety *Agaricus bitorquus*.

The product was irradiated with 0, 100, 150 and 200 krad gamma rays and stored at 10 °C; humidity 85%.

This experiment showed that external quality and colour were preserved at 10 °C, but that the product was sensitized for "growth" and "open caps". Irradiation considerably delayed this process.

The optimal dose was approx. 200 krad and at this dose, the discolouring of the gills was delayed too.

Irradiation of prepacked onions

The quality of cut onions is strongly affected by microbiological decay during storage, transport, marketing and by browning after cooking. In collaboration with the Sprenger Institute the influence of irradiation and packaging on the keeping quality of prepacked cut onions during storage was studied. The product was irradiated with 0, 50, 75, 100 and 150 krad gamma rays and stored at 15 °C.

The results showed that a dose of 100 and 150 krad lengthened the shelflife with 50%. At a dose of ≥ 100 krad the number of micro-organism was reduced by a factor of $10^3 - 10^4$.

In the non-perforated bags the browning after cooking was prevented by an O₂ percentage lower than 3%. In the perforated bags the unirradiated onions discoloured considerably after cooking; an irradiation dose of 75 krad already prevented this browning. The organoleptic properties of the onions were not affected by an irradiation dose of ≤ 100 krad.

Irradiation of prepacked endive.

The experiments of last year were repeated and extended in collaboration with the Sprenger Institute. Much attention has been paid to packaging, microbiology and organoleptic tests. The prepacked product was irradiated with 0, 100 and 120 krad gamma rays and stored at 12 °C.

In combination with irradiation the best quality was found in the non-perforated bags of 0,02 mm thickness. Microbiological research showed that at a dose ≥ 100 krad the total viable count (PCA-medium) and the Enterobacteriaceae (VRB-medium) were reduced by a factor of 10^4 , resulting again in a complete elimination of the Enterobacteriaceae. Organoleptic tests proved that a dose of 120 krad gave no off-flavours.

Publications:

Langerak, D.Is. The influence of irradiation and packaging upon the keeping quality of fresh mushrooms.
Mushroom Science VIII. Proceedings of the Eighth International Congress on Mushrooms Science, 221-230 (1972).

Langerak, D.Is. De toepassing van straling bij de bewaring van groenten en fruit.
Voedingsmiddelentechnologie 3, Nr. 34/35, 182-184 (1972).

In Press:

Langerak, D.Is. and Bruurs M.F.L. Preliminary study concerning the influence of combined heat and radiation treatment on the quality of some horticultural products.
Acta Alimentaria Academiae Scientiarum Hungaricae.

Annual report 1972 of the Association EURATOM-ITAL.

Hoofd van het team en wetenschappelijke medewerkers:

K.J.A. Wijnands-Stäb, C. van Heemert, M.J. Frissel.

Titel van het project: Radiobiological and genetic studies on insects.

Beschrijving van de resultaten:

1. Study of genetical processes induced by irradiation in the onionfly *Hylemya antiqua* Meigen.

With the aim of finishing in 1972 the preliminary study of the use of chromosomal translocations for genetic control of the onionfly, two series of irradiation experiments were carried out. Some of the conditions, which influence the induction of chromosomal rearrangements were again considered: sex and age of the insect, storage period of the pupae, radiation dose (0.25 and 0.5 krad fast neutrons; 1.0 and 1.5 krad X-rays). Obtained results concern: immediate effect on the fertility (% egg hatch) of the treated generation, data on the % egg hatch of the F₁ in the first back-cross, information concerning the following generations of some strains, suspect of carrying a chromosomal rearrangement characteristic. The cytogenetic analysis of the interesting strains (collaboration with Ir. C. van Heemert, Dept. of Genetics, Agric. Univ. at Wageningen), as well as the necessary back-crosses are not yet finished.

The following preliminary conclusions may be drawn:

- Storage of pupae over a long period reduced the sensibility of males to immediate effects of irradiation (predominantly dominant lethals).
- Values of % egg hatch after irradiation vary irregularly. For males 0.5 krad of fast neutrons give 3.9% egg hatch, 0.25 krad of fast neutrons give 7.9 to 12.0% egg hatch; values for 1.5 krad X-rays vary from 2.6 up to 7.6% egg hatch. For females 0.5 krad of fast neutrons give 10.1% egg hatch and 0.25 krad of fast neutrons give 7.7 % egg hatch.
Presumably the dose-effect curve at these doses is rather flat, as well for fast neutrons as for X-rays.
- Comparing the data for irradiated females with previous ones, it is obvious that irradiation at an age of 7 days is not so disastrous for fecundity as irradiation at the first day after emergence.
- Doses above 1.0 krad of X-rays, or an equivalent dose of fast neutrons (+ 0.25 krad) show in cytogenetic analysis always complicated

rearrangements. (Personal communication of C. van Heemert).

It is therefore advisable to use doses below 1.0 krad of X-rays for induction of simple chromosomal rearrangements, such as reciprocal translocations.

2. Simulation of the population dynamics of the onionfly in the field.

(in collaboration with Dr. M.J. Frissel and using ecological data from the onion-fly team (J. Ticheler, M. Loosjes, Inst. of Phytopath. Res. at Wageningen)).

The purpose of the first model was theoretic evaluation of different methods for genetic control in such a complex system as the natural environment.

A flow chart of this model, in which the developmental stages are extensively considered in relation to the crop, is presented in fig. 1. It contains various density dependent regulations, e.g. on the egg production of females and survival of larvae, and seasonal influences like temperature sensitive development of stages such as aging of females, larvae and diapause induction.

This first model has been simplified because many parameters were not known, and a simple programme was as useful for simulation of fluctuations of density in the course of several years, and of genetic load on the population, in case of release of translocations (fig. 2).

A second model was especially made to simulate for onionfly the release of two independent translocation stocks, a situation previously described by Curtis and Robinson (written with the advise of Dr. C.F. Curtis).

Table 1 represents some results of the simulation. The two independent translocations can be released into the population as two homozygote strains (R and T) in a mutual ratio of 1:1 (R+T 1:1 in table 1) or as double heterozygotes (v in table 1). The ratio of the numbers released to the natural population is another parameter. The genetic load (\bar{w}) indicates the reduction of the total egg hatch in all possible mating combinations. The release of translocations at different release ratios is compared with release of steriles. Releases may be repeated for several generations, in which case the addition of a number of partial fertile flies has to be considered as a negative factor.

3. Experimental approach of the release of translocation strains in a normal population.

For better understanding of the effect of release of translocation strains into a normal population, experiments with *Drosophila melanogaster* in population cages were carried out at the Inst. of Genetics, Univ. of Utrecht in cooperation with Prof. Dr. W. Scharloo. It turned out that the variation in these cages was even larger than in irradiation experiments on the onionfly.

Without preceding standardization of the stocks to be used, manipulation of *Drosophila* as an insect model for studies of the population growth of mixtures with translocations is unreliable. In the experiments done the influence of released translocations on the growth of the population was small.

Publication :

Annual report 1972 of the Association EURATOM-ITAL.

Table 1 - Genetic load on population of onionflies after application of chromosomal translocations.
 Growth factor 4 per generation. Original population 10^4 onion flies.

	type of application	release ratio	\bar{w} 1st generation	\bar{w} 2nd generation	\bar{w} 3rd generation	\bar{w} 9th generation	\bar{w} 10th generation	population after 11 generations	total release translocations
	2 homozygous strains								
Application only once	R+T 1:1	0.5	1.0	0.56	0.63	0.98	0.99	1.0×10^{10}	0.5×10^4
	"	1	1.0	0.45	0.48	0.94	0.97	2.7×10^9	10^4
	"	4	1.0	0.38	0.36	0.45	0.51	3.0×10^7	4.10^4
	"	10	1.0	0.38	0.36	0.35	0.35	1.4×10^7	10^5
	"	0.5	0.57	0.82	0.91	1.0	1.0	2.4×10^{10}	0.5×10^4
	double hetero zygote(v)								
	"	1	0.40	0.69	0.81	1.0	1.0	1.5×10^{10}	10^4
	"	4	0.19	0.41	0.50	0.97	0.98	3.2×10^9	4.10^4
	"	10	0.14	0.32	0.37	0.82	0.89	2.3×10^8	10^5
	steriles	10	0.09	1.0	1.0	1.0	1.0	3.8×10^9	10^5
	none	-	1.0	1.0	1.0	1.0	1.0	4.2×10^{10}	-
Application to each generation	R+T 1:1	10	1.0	0.42	0.41	0.41	0.41	2.2×10^8	1.9×10^7
	v	10	0.14	0.17	0.18	0.21	0.21	3.4×10^6	4.2×10^6
	steriles	0.5	0.67	0.67	0.67	0.67	0.67	4.8×10^8	5.5×10^7
	"	1	0.50	0.50	0.50	0.50	0.50	2.0×10^7	1.0×10^7
	"	4	0.20	0.20	0.20	0.20	0.20	860	1.7×10^5
	"	10	0.09	0.09	0.09	0.09	0.09	0	1.6×10^5

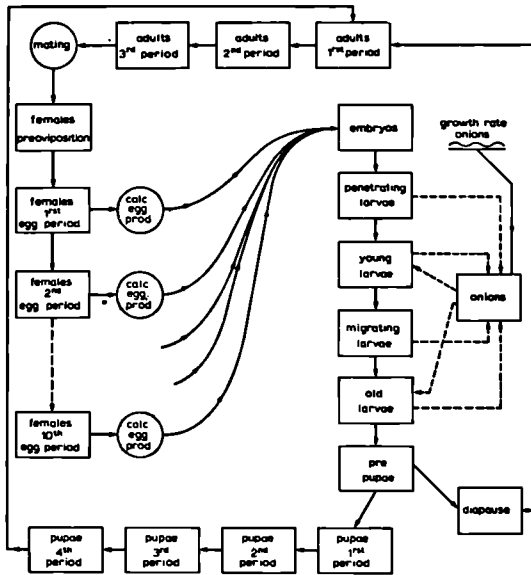


Fig. 1 - Flow chart population dynamics onionfly.

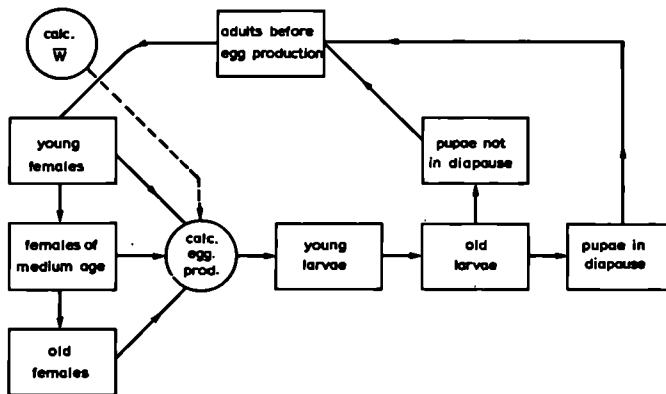


Fig. 2.- Flow chart simplified population dynamics onionfly.

\bar{W} stands for the input of a certain genetic load such as is caused by release of translocations.

Hoofd van het team en wetenschappelijke medewerkers:

D. Snieder.

Titel van het project: Genetical control of Adoxophyes orana F.R.

Beschrijving van de resultaten:

Figure 1 gives a summary of the average percentages egg hatch, when male and female moths have been irradiated with substerilizing dosages of X-rays and their progenies have been outcrossed in succeeding generations with untreated moths.

Female moths have been irradiated with 2 1/2 and 5 krad X-rays, male moths with 7 1/2, 10, 15 and 22 1/2 krad.

It appears that the F1-progeny from males irradiated with 22 1/2 krad and crossed with untreated females produces no larvae of the B1-generation. The line from males irradiated with 15 krad is not able to produce larvae of the B2-generation. These two lines therefore die out.

The progeny from the other irradiated moths shows, after two or three generations of outcrossing, a normal percentage egg hatch. Therefore a rapid recovery from the incurred damage takes place. It is remarkable that the progeny from irradiated females has recovered the normal percentage egg hatch one generation earlier than the progeny from irradiated males. Figure 1 shows that the F1 from irradiated females has a percentage egg hatch that equals or exceeds that of the parent generation; the F1 from irradiated males however has a percentage egg hatch which is obviously lower than that of the parent generation. In other words the repair of the radiation damage starts in the first generation after the irradiation of the females, and in the second generation after the irradiation of the males.

To explain this difference, first some information about cytological observations concerning visible chromosomal aberrations in meiotic cells of the tests of L5-larvae has to be given. It was found that the aberrations induced by irradiation have disappeared almost completely from the larvae of the B1-generation. Because a high percentage of the observed aberrations looked like simple reciprocal trans-locations, it is accepted

that the rapid and almost complete disappearance of the visible aberrations is not caused by the fact that, as a result of the structural mutations, no fertile reproductive cells can be formed, but by the production by means of the radiation of a considerable number of fragments. Due to a lack of chiasmata, these fragments can not orientate themselves in a right way during meiosis and therefore hinder a correct distribution of the chromosomal material.

It is further accepted that the holokinetic structure of the chromosomes of Lepidoptera permits fragments and structural mutations to go through the mitotic divisions. In the meiotic divisions, an important selection occurs (see above), resulting in a great number of sterile reproductive cells. In the lines from irradiated males the first meiosis after irradiation takes place in the 5th larval stage of the F1-larvae; in the lines from irradiated females this occurs right after the irradiation. Consequently there is a difference of one generation in recovery from radiation damage.

The rapid disappearance of the induced chromosomal aberrations means that the dosage of radiation has to be reduced for induction of translocations, useful in a genetic control of insect pests. We find ourselves than in the range used to induce translocations in the more radiosensitive Diptera. This would mean that, with respect to the induction of breaks and fragments, the chromosomes of Lepidoptera and Diptera react in a similar way to radiation. The differences in radiosensitivity between both orders are due to quite different processes, which assert themselves after the primary action of the radiation.

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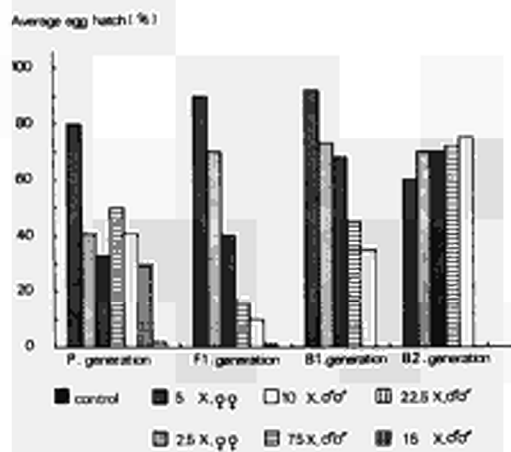


Fig. 1 Average percentages egg hatch in succeeding generations after irradiation of male and female moths of Adoxophyes orana with several dosages of X-rays.

Resultaten van het project No. 29.

Hoofd van het team en wetenschappelijke medewerkers:

A.M. Feldmann.

Titel van het project: Genetical and radiobiological studies on
Tetranychus urticae C.L. Koch.

Beschrijving van de resultaten:

Treated subjects.

- I. Inducing effects such as dominant lethals, recessive lethals and structural chromosome mutations, at low doses of X-ray irradiation.
 1. Induction of these effects by irradiation of 1 day old adult males.
 2. Induction of these effects by irradiation of 1 day old adult females.
- II. Initial research on the repairmechanisms of the irradiation effects.
- III. Induction of temperature sensitive mutations.

- I. 1. The percentages of the dominant lethals, induced by the different applicated doses are ranging from 0% at 1 krad X-rays up to 59% at 8 krad. It is found that the effects of recessive lethals and structural chromosome mutations, which are expressed in the F₂ (as far as the diploids are concerned) are difficult to distinguish from each other and are apparently often combined in the same organism.

A method was developed by the aid of which recessive lethals can be distinguished from mutations with the characteristics of structural chromosome mutations c.q. translocations. By determining the eggmortality, respectively in the haploid eggbatch and in the haplo-diploid eggbatch, both produced by the same F₁-female, the influence of fertilization, by which recessive factors are complementated in contrast with the effects of translocations, can be followed. In this way a range of mortality-levels are compiled in a frequency-diagram (see fig. 1) for the applicated doses of X-rays (0.0; 0.5; 1.0; 1.5; 2.0; 4.0 and 8.0 krad).

It is supposed, that, for genetical control, the group γ is very important, because the F₁ females, belonging to this group, are heterozygous for a factor, with the characteristics of a structural chromosome mutation. A preliminary conclusion from the results in Fig. 1 is that translocations are induced at the highest frequency,

at doses between 1.5 and 0.5 krad X-rays, applicated on 1 day old adult males of *T. urticae*.

I.2. Up till now, only the effects, caused by the irradiation of 1 day old virgins of *Tetranychus urticae* Koch with 1.0; 2,0 and 8.0 krad X-rays are studied. Striking differences exist with the results under I.1. Virgins, irradiated with 8 krad X-rays produce after fertilization an F_1 , in which 89% dominant lethals are found. The adult F_1 -females, however, have a normal fertility! Untreated females, crossed with F_1 males, descending from parents, of which the female was irradiated with 8 krad X-rays, produce a progeny with a normal mortality.

II.1. By crossing of irradiated males on successive days with new groups of virgins, it is possible to study the correlation of time passed after irradiation, with the effects induced in the sperm. When males, 0 - 24 hr after irradiation with 1,5 krad X-rays, are crossed with untreated virgins, 12% dominant lethals are produced in the F_1 . This percentage is lowered to 4% when males, 48-72 hr after irradiation with 1,5 krad X-rays, are crossed to virgins. No dominant lethals are found when males 100 -124 hr before the mating act are irradiated.

Also in the F_2 , indications of irradiation repair mechanisms are found (see fig. 2) T is the period between the irradiationmoment and the moment of crossing. When $T = 0 - 24$ hr, an F_1 is produced of which 18% of the F_1 -females are sterile (α_1^2).

When $T = 48 - 72$ hr, only 4% of the F_1 -females are sterile. No sterile females are found when $T = 100 - 124$ hrs. The correlation of time T with the frequency of E , is positive and very pronounced.

II.2. In contrast with II.1 no differences are found in the fertility-patterns of F_1 -females, descending from eggatches produced respectively 24 - 48 hr and 134 - 158 hr after crossing with the irradiated male parents.

From the results of II.1 and II.2 it seems to be very probable that the correlation between T and the changes in the fertility patterns (see II.1) is not caused by a repair mechanism s.s. but possibly by a difference in radiosensitivity of the different developmental stages of the spermatids. The results, however, can also be explained by postulating a retard of the development of gametes with genetical irradiation damage, followed by resorption of these cells. The result of this process is a positive selection of gametes with slight or none genetical damage. It

is also possible, that repair mechanisms are only active in developmental stages of the spermatids and not in mature sperm. To verify these hypotheses much more research is needed.

III. The subject of developing temperature-sensitive mutations in *Tetranychus urticae* Koch is in a preliminary stage. Experimental conditions as the right dose and temperature discrimination have to be specified.

For isolating temperature sensitive mutations, the method of Smith, R.H. (see pag. 462 of the IAEA Symposium on Sterility Principle for Insect Control or Eradication, Vienna, 1971) is modified and adapted to the arrhenotokous mode of reproduction of *Tetranychus urticae* Koch. By comparing the splitting proportions of the albino-mutation and its wild-type allele in backcross I at permissive and restrictive temperatures, the occurrence of dominant- or recessive temperature sensitive mutations can be noted.

It is expected, however, that the yield of the method used, will be very low. This is caused by the high recombination frequency, which is characteristic of *Tetranychus*; only temperaturesensitive mutations with a very high degree of linkage with the used albino-locus can be observed.

Publication :

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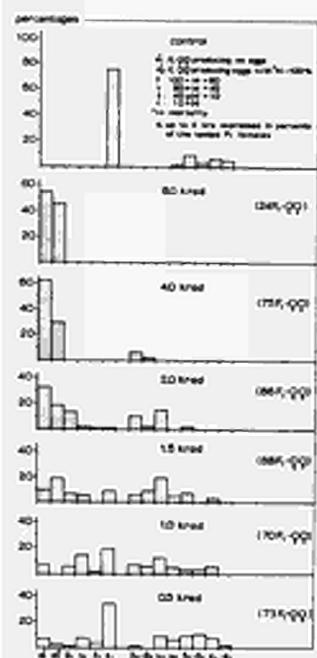


Fig. 1. Fertility pattern of F_1 females of *Tetranychus urticae* Koch as found in the haploid F_1 , and haplo-diploid backcross I. Of the parents, the male fs irradiated with X-rays.

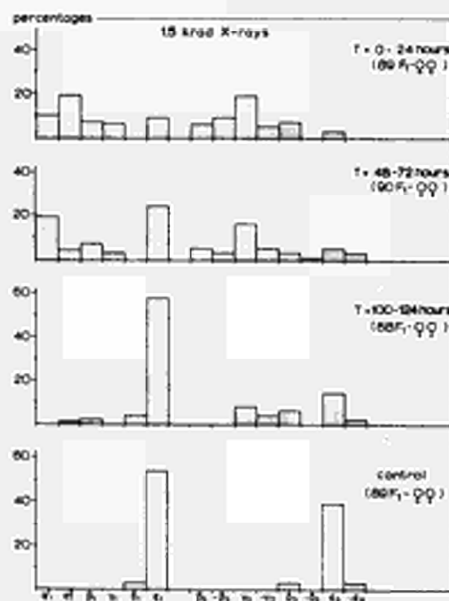


Fig. 2. Fertility pattern of F_1 -females, descending from a cross, of which the male parents T-hours before mating were irradiated with 1.5 krad X-rays.

Resultaten van het project No. 30

Hoofd van het team en wetenschappelijke medewerkers:

G. Sauer, S.C.v.d. Geijn, G.P. Mix

Titel van het project: Measurement and localization of biological processes of tissue, cellular and subcellular level.

Beschrijving van de resultaten:

1. Methodology for microautoradiographic purposes

Freeze-drying and freeze-substitution for dehydration of various plant tissues were again compared. It was confirmed that the rapid freezing, causing disruption of the tissue, is the main disadvantage of these procedures, as compared to dehydration at room temperature. Satisfactory results were obtained with the butylmethacrylate and Epon-Eraldite embedding agents in some cases, mainly after freeze-substitution. Successful preparation of the tissue-slices mainly depends, of course, on the quality of the dehydration and embedding processes. The experience up till now is that the preparation procedure for the material to be cut in thin slices and used in track-autoradiography, has to be adapted, each time a different type of plant tissue is considered.

During the past year, research efforts have been concentrated on one major problem:

2. Calcium translocation into seeds and fruits

a. Materials and methods

In this respect, the following techniques were used: micro-autoradiography, macroautoradiography, 'in vivo' counting by semiconductor detectors and liquid scintillation counting.

The experimental material was: fruits of bean plants (Phaseolus vulgaris L.) and fruitstems of paprika (Capsicum annuum L.). Plants of both species were grown in a climate controlled (25000 lux; 16 hrs day; 25°C; rel.humid. 70%) growth chamber on an aerated complete nutrient solution. In the different experiments, 50 to 800 $\mu\text{Ci } ^{45}\text{Ca}$ were applied in the nutrient solution of bean plants at the fruiting stage, whereas 30 $\mu\text{Ci } ^{45}\text{Ca}$ in a 1 M Ca (NO_3)₂ solution were injected in the paprika fruit stem at 5 cm distance from the fruit-insertion.

For micro- and macro-autoradiographic purposes samples were harvested at different time intervals after the ^{45}Ca -application. After freezing, these samples were dehydrated, either by freeze-drying or freeze-substitution and embedded in methacrylate, epon-

araldite or paraffin wax. For microautoradiography Ilford K5-emulsion was applied and developed after 3 days exposure and gold-sensitization (Rechenmann et al. C.R. Acad. Sc. D 269: 1525-1527 (1971)). Kodak X-ray film was exposed for 7 days to the irradiation by macroautoradiographic samples (mainly transverse sections through the bean fruit) and developed in the usual way. 'In vivo' semiconductor detector counting has been described in previous reports (see also report 8 and 34). Liquid scintillation counting was done on ashed (500°C) samples dissolved in 0.1 N HCl.

b. Evaluation of the results

Paprika fruitstems.

The preparations for the microautoradiography were strongly damaged due to the bad penetration of the embedding agents in the partly ligneous material. The microautoradiographs from samples harvested 4 to 26 h after injection of the ^{45}Ca solution show a rather uniform distribution over the different tissues, even after only 4 h of application.

On a cellular level the calcium was found mainly inside the cells and less in the cell walls. No conclusions could be drawn with respect to the amount of calcium present in relation to time after application, due to the variation between the individual samples.

Bean fruits.

Macroautoradiographs were made of cross sections of fruits harvested 5 to 25 h after root application. The penetration of the calcium in the fruit takes place through both vascular bundles. From these sites a slow lateral distribution in the fruitwall is observed during the first 25 hours. The rate of transport in the seed stem is comparable to that in the fruitwall.

The ratio between the ^{45}Ca concentration in the vascular bundles at the dorsal and the ventral side appears to be a function of the seed size. This was measured by semiconductor detectors and confirmed by liquid scintillation counting. At small seed sizes (<10 mg d.w.) this ratio is about 1. The relative concentration at the dorsal side is increasing significantly with seed size.

The countrate measured at the vascular bundle by the semi-

conductor detectors is representative of the actual amount of ^{45}Ca at this position in a highly significant way. This can be ascribed to the fact that the internal distribution in a superficial layer (< 300 μm deep) near the vascular bundle is rather stable in time and also comparable in the different fruits.

Microautoradiographs obtained from samples harvested 17 h after root application show that nearly all of the ^{45}Ca is concentrated in a narrow cell layer where bark and phloem meet (bundle sheath). Due to this very high concentration, causing a local overexposure, no track autoradiographic localization on a cellular level was possible at this moment. For this purpose preparations with a lower amount of activity should be used.

Publications:

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G.P. Mix, Anwendung der Mikroautoradiographie und der Semikonduktor-Detektor-Messung zum Problem der Ca-Ein- bzw. Umlagerung in Früchten. Institut für Pflanzenernährung, Berlin.

Resultaten van het project No. 32

Hoofd van het team en wetenschappelijke medewerkers:

J.F. Stoutjesdijk.

Titel van het project: The propagation of nuclear methods in biology and agriculture.

Beschrijving van de resultaten:

1. Collaboration with other institutes

- .1 Investigations about the dietary compounds that prevent iron retention (in collaboration with Ir. A.B. Cramwinckel, Department of Food and Nutrition, Agricultural University, Wageningen).

Originally it was planned to enrich the foods of the rats, used in the experiments, with vegetable food and hemoglobine, both labelled with ^{59}Fe . However, both labelling common cress (Lepidium sativum L.) by growing it in a ^{59}Fe -spiked medium, and injection of the radio isotope interveniously into rabbits, proved to give only small amounts of ^{59}Fe labelled material and, with the available equipment and personnel, it was not possible to obtain enough labelled material for reliable results in the retention experiments.

Different foods or their components (egg, buttermilk, milk, bread, coconut fat, maize oil and beef fat) were studied in vitro and in vivo under comparable conditions to examine whether they had an inhibiting effect on the iron retention by the gut. In the experiments in vitro, performed at the institute, a weak solution of ^{59}Fe -ferrous or ferric citrate was brought into contact with each of the foodstuffs in a sealed bag of semipermeable material. Unlike large molecules and hydrophobe fats, small particles like ferrous and ferric ions are not stopped by this material. The radioactivity measured outside the enclosed space was used to determine the fixative capacity for these ions of the particular food. By using radioactive iron it was possible to measure quickly and with great accuracy very small amounts of ferrous and ferric ions.

The in vivo experiments have been carried out with rats at the Department fo Food and Nutrition of the Agricultural

University of Wageningen. Iron retention has been determined by measuring the total animal radioactivity in a whole body counter immediately after the induction of the food with a ^{59}Fe -ferric citrate solution into the stomach of the rat and again after two weeks.

With the exception of egg, there was little correlation between experiments made in vitro and in vivo. Egg inhibited iron retention, which effect was also found for milk in vitro. No effect was found with bread in vitro. The fats had different effects in vitro: maize oil lowered iron levels in rats, beef fat raised these levels. Coconut fat was intermediate. Plans for a double tracer experiment with ^{55}Fe and ^{59}Fe with human volunteers have not been carried out after a negative advice by the Ministry of Public Health and Environmental Hygiene.

The results of the investigations have been embodied in the doctor's thesis of Cramwinckel: 'Dietary components that prevent iron retention', Wageningen (1972).

2. Determination of algal growth in surface water. Collaboration with Mr. W. Eenboom of the Governmental Service for the IJssellake polders.

Algal growth is a measure for the pollution of surface water; it can be measured by determination of the O_2 produced by photosynthesis, but in heavily polluted water this method is not longer feasible as the O_2 may directly escapes from the water. Also a radiological method with ^{14}C is in use: ^{14}C -carbonate is added to a sample of the water in a bottle and the ^{14}C -content of filtered off algae is measured after a certain time of illumination. The preparation of stable and reproducible samples for liquid scintillation counting of the ^{14}C gave difficulties which could not yet be overcome. Further work is in progress.

2. Work for the C.C.R.A. (Co-ordination Commission Radioactivity Measurement)

My work, as technical secretary, consisted of:

- preparation of the annual report 1971;
- revision of the National Measuring Programme for controlling the environment of nuclear reactors;

- membership of a commission of the Ministry of Public Health and Environmental Hygiene for developing of standards for emergency situations after accidents in nuclear installations;
- attending the meetings.

3. Courses

- .1 A general radioisotope course was organized from March 6 to 24, for 21 participants, of whom 3 members of the Association's personnel.
- .2 Two Liquid Scintillation Counting courses were organized from November 20 till December 1 and December 4 till 15 with 19 and 21 participants respectively. Among them were two Belgian participants and 10 members of the Association's personnel.
- .3 A course on Health Physics was proposed but it could not be organized due to insufficient participants.

Hoofd van het team en wetenschappelijke medewerkers:

J.G. de Swart, J.F. Stoutjesdijk.

Titel van het project: Development of nuclear techniques and related instrumentations for biological and agricultural research.

Beschrijving van de resultaten:

The first part of this report is dealing with work directly related to the Association's programme (Items 1, 2, 3 and 4); the items 4, 5, 6 and 7, are related to work carried out in cooperation with Institutes outside the Association.

1. Improved soil column scanner for water movement studies in soils - Reiniger (soils group) and Stroosnijder (Dept. of Soils and Fertilizers, Agric. Univ., Wageningen).

The unknown packing of soils in soil columns is an important disturbing factor in all measurements. Using the different mass-absorption coefficients of gammas from two sources (^{241}Am (60 keV) and ^{137}Cs (661 keV)), measured simultaneously, it should be possible to calculate the moisture content independently of the differences in soil density.

The measurement system consists of a scanning mechanism, 2 spectrometer channels with their own properly collimated scintillation detectors, 1 digital read-out unit, a printer-puncher and the interface control unit.

The instrument (Fig. 1) has now been realized and tested in an experiment on a swelling soil. See also the report of project 2.

2. A data collection system, the development of which was started will facilitate the solution of actual measurement problems. This system collects both digital and analog data and registration is done by punch tape, magnetic tape or parallel printer.

3. The development of a fast peak detector, to be used with the atomic absorption spectrophotometer equipped with a graphite atomizer, is in progress. A punch tape read out of the modular system type has been built and will be used in relation to a sample changer which has to be developed for the atomic absorption spectrophotometer.

4. For protection of maintenance workers during nights and weekends an automatic alarm system was developed. The system operates via a radiotranceiver combined with a semaphore terminal in such a way, that the operator will be called automatically at fixed time intervals. If he does not react on these calls, the on duty Health Physics man will get an alarm via his semaphore. The system is planned to be operational beginning 1973.

5. Measurement of density variations of flowing granular material in silo systems - Van Zuilichem, Dept. for Food Technology, Agric. University, Wageningen.

A method was worked out to find the influence of the outlet shape of an elevator system on the density of the flowing material near this outlet. Attenuation of a properly collimated γ -beam (50 mCi ^{137}Cs -source) by different granular materials flowing in a model of an elevator system is measured at different places in the model by a scintillation detector followed by a one-channel γ -spectrometer system.

The measurements are transformed by a computer program in a density plot.

The method works satisfactorily and one of the results is that the 'arch of free fall'-theory which is generally used in the analysis of the process, seems to fail. Working out of another mathematical approximation of the flow-process in silos is in progress.

6. Velocity measurement of granular material in an elevator system - Van Zuilichem, Stolp, Dept. for Food Technology, Agric. Univ., Wageningen.

The material is vertically transported in an air stream over more than 20 m with speeds of 15 to 30 m/sec. The velocity of the solid material depends on different factors, e.g. porosity, tube diameter etc. A shot of activated material is brought into the tube, during a normal transport process, and the time the activity needs to reach a detector at a certain position along the tube is measured. Because of the high speed of the material, the measurement is rather short (m sec), c.q. the count rate needs to be high and the time resolution extremely good.

Therefore a multichannel analyzer is used in multi scaler mode.

The address advance (time base) is driven by a pulse train with a precisely known frequency, while the start of the pulse train is synchronized with the sample shot.

A large number of measurements is now available. The measurements will be finished during summer 1973. The velocity data have still to be analysed in relation to other information concerning the overall transport process in the elevator systems.

7. Moisture content measurements in plant leaves - Schildwacht, Botanical laboratory of the State University, Utrecht.

The system, using the absorption of soft β radiation (e.g. ^{45}Ca and G.M. detection is very similar to earlier reported devices.

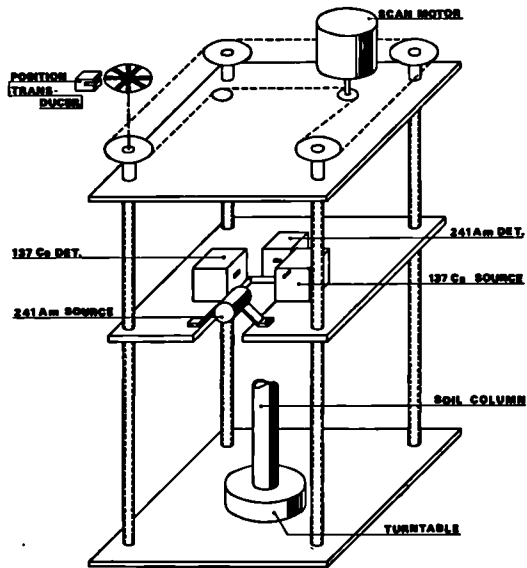


Fig. 1. Schematic representation of soil column scanner permitting simultaneous measurement of water movement and soil density.

Publication :

Annual Report 1972, of the Association EURATOM-ITAL.

Hoofd van het team en wetenschappelijke medewerkers:

S.C. van de Geijn.

Titel van het project: Development and initial application of semi-conductor detector assembly for biological research.

Beschrijving van de resultaten:

β -Sprectrometry

A. In earlier reports a method was presented for the in-depth localization of β -emitters based upon the measured shift in maximum energy. The method was shown to be applicable to isotopes having a maximum energy ranging from 154 keV (^{14}C) to 1710 keV (^{32}P). A further study has been made which can be summarized in the following points.

1. Fjeld and Verghese (Nucl. Instr. and Meth. 93, (1971) 573)) recently published results, obtained by applying the method to the 514 keV β -spectrum of ^{137}Cs , which are deviating from theory. The deviation was ascribed to distortion of the spectrum due to the preferential attenuation of the lower energy electrons, and the reduced stopping power of higher energy electrons.

In order to investigate the existing discrepancy, measurements were done of the dependence of the different components of the ^{137}Cs spectrum on absorber thickness. The results show, that the deviation is due to the incomplete subtraction of the low energy tail of the conversion electron spectrum at high absorber thicknesses, and therefore does not present a fundamental limitation to the method.

2. The survey of the range of applicability of the method has been completed by a study of isotopes, of importance in biology, having complex decay schemes. For this purpose ^{22}Na and $^{89}\text{Sr} + ^{90}\text{Sr}$ were selected.

The small-volume semiconductor charged particle detectors have very low sensitivity to γ -radiation. A correction of the beta spectrum is necessary however, if the background spectrum interferes with it in the energy region generally used for the determination of the maximum energy (70 - 90 % of E_{max} , the maximum transmitted beta-energy). The same applies to isotopes

exhibiting a branched beta decay or to a mixture of beta emitters. The magnitude of the background contribution (gamma rays or high energy beta rays) can be determined using the specific properties of the spectrum. This has been worked out for ^{22}Na and for ^{89}Sr contaminated with about 5 % ^{90}Sr .

The spectrum of ^{89}Sr (β^- , $E_{\text{max}} = 1463$ keV) is influenced in the maximum energy region by the beta spectrum of ^{90}Y (β^- , $E_{\text{max}} = 2270$ keV) which is the daughter product of ^{90}Sr (β^- , $E_{\text{max}} = 546$ keV). The very high energy of ^{90}Y permits the determination of its contribution in a region well above the maximum energy of ^{89}Sr . The results of the extrapolation calculations, after a background subtraction, are shown in Fig 1. The shape factor has been included in the calculations to restore the linearity of the Kurie plot of this unique first forbidden spectrum.

The β^+ decay of ^{22}Na ($E_{\text{max}} = 545$ keV) is accompanied by a 1275 keV γ -ray and by 511 keV annihilation radiation. The Compton edge of the annihilation radiation spectrum, at 341 keV, approaches the energy region generally used for the determination of the maximum energy of the beta spectrum. A background subtraction is performed assuming a constant ratio between the number of 1275 keV γ -rays and 511 keV annihilation quanta causing a Compton electron background. The magnitude of the contribution is determined, using a least squares fit between the uncorrected beta spectrum and a reference gamma background in an energy region above the maximum beta energy. The results obtained by applying the method to the corrected beta spectrum are shown in Fig 1.

3. The maximum depth for which localization is possible strongly depends on the maximum energy of the beta particles.

For ^{32}P it is found to be about 3500 μm ($\rho = 1 \text{ g/cm}^3$) whereas for ^{14}C the maximum distance is 200 μm as reported before.

4. One of the limitations of the method as described before is caused by the required linearity of the Kurie plot in the high energy region. This linearity can be expected only if a relatively thin layer of labeled material is present in an otherwise non-labeled subject. This problem has been partly overcome, using a non-linear extrapolation. A further improvement is obtained by a modification of the Kurie transformation, which consists of:

$$[N(E) / \{ k(p + qE) \}]^m$$

where:

$N(E)$ = the spectral intensity at the energy E ,

k, p, q = constants.

m chosen to give a maximum linearity of the plot, instead of $m = 0.5$ in the original Kurie transformation.

By this approach ^{45}Ca -localization is possible with an accuracy of approx. $15 \mu\text{m}$ ($\rho = 1 \text{ g/cm}^3$) and this localization is independent of the internal distribution beyond the top layer.

B. The method for the determination of the in-depth distribution has been further tested.

1. For ^{45}Ca the accuracy of the method is estimated to be about $\pm 50 \mu\text{m}$, whereas the amount of activity in the top layers can be determined with an accuracy of $\pm 20 \%$. The accuracy of the calculated amount of activity in the deeper layers is strongly dependent on the amount in the top layers. This is due to the important reduction in countrate with increasing thicknesses. The maximum depth about which information can be obtained is approx. $300 \mu\text{m}$.

2. A biological application is actually in progress (in collaboration with G.P. Mix, Institut für Pflanzenernährung, Berlin). This study concerns the uptake and accumulation of calcium, in bean fruits (Phaseolus vulgaris L., var Saxer) after root application. The comparison of the calculated distribution with the results obtained by micro-autoradiography is promising. Further experiments are in progress.

3. An automatic sample changer has been developed which facilitates the measurement of the reference spectra needed for the determination of the in-depth distribution of ^{45}Ca . This apparatus has been designed to operate in an interactive mode with either of two multi-channel analyzer systems. It enables the automatic measurement of a series of spectra either through different absorbers, or of sources of different composition (maximum 6). A more efficient use of the equipment is realized in this way.

Publications:

Annual report 1972 of the Association EURATOM-ITAL.

Publications: (submitted to the Editor of Nucl. Instr. and Meth.)

Van de Geijn, S.C. On the determination of electron energy losses in absorbers; A study of the ^{137}Cs spectrum.

Van de Geijn, S.C. In-depth localization of beta-emitting isotopes; Dependence of the range of applicability upon maximum energy and complexity of the spectrum.

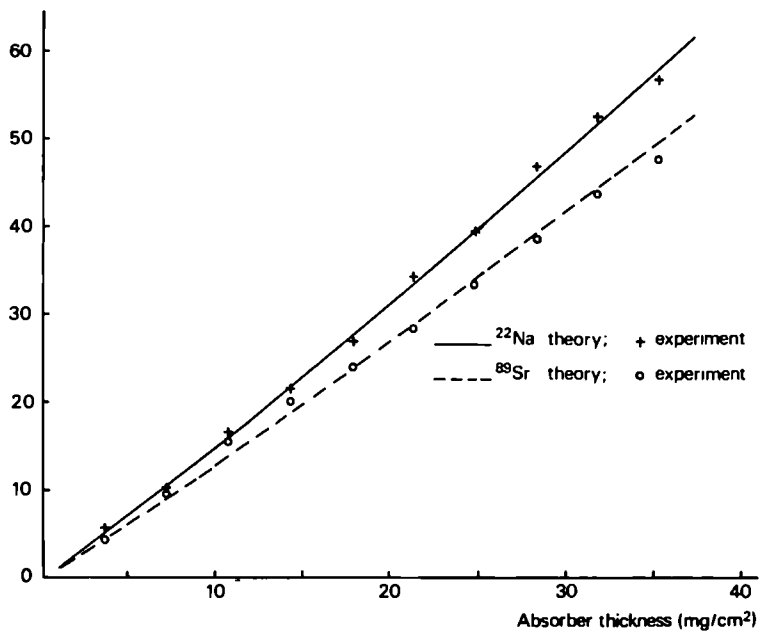


Fig. 1. Theoretical energy loss of electrons in polyester layers as a function of absorber thickness. The upper curve is obtained for ^{22}Na electrons ($E_{\text{max}} = 545$ keV); the lower curve applies to the beta spectrum of ^{89}Sr ($E_{\text{max}} = 1463$ keV). The points represent the corresponding experimental values.

Hoofd van het team en wetenschappelijke medewerkers:

P. Poelstra, N. v.d. Klugt.

Titel van het project: Neutron activation analysis.

Beschrijving van de resultaten:

During the past years the method itself (preparation of biological samples etc.), a computer programme for identification, calculation of the concentration of all elements, also present in the reference standard, and finally a computer programme for the administrative elaboration of the samples have been worked out and adapted to the facilities available at the Association's Institute (e.g. Reactor Barn).

For control, the method has been applied in 1972 to the solution of a few practical problems.

1. On request of the Soils Department of the Agricultural University at Wageningen about 500 samples from Dutch Guyana were analysed, for Fe, Co, Cu, Zn and Mn. The samples consisted of soil, biological material and soil solution. The results will be available in a thesis by Ir. Scheltema.
2. In behalf of the Ministry of Agriculture and Fisheries a measurement programme was set up for the determination of a number of heavy metals in fish products. Sampling is done by the Netherlands Institute for Fisheries Research, IJmuiden. About 10 species are sampled every three months and analysed for Cu, Fe, Zn, Mn, Mo, Cr, As, Cd and Hg.
The analysis is done instrumentally for all elements, except Cd, As and Hg. These metals are isolated after activation and destruction of the sample. Hg and As are successively separated from Cd by distillation, first Hg and then As. Cd remains in the residue and is isolated by precipitation as Cd-sulphide. The As is precipitated with NH_4 -hypophosphate and Hg is isolated by electrolysis on a gold foil.
3. On behalf of project 20, bacterial spores were irradiated for the identification of the metals present. Fig. 1 shows the γ -spectrum from such spores after different decay periods. The

sample was irradiated for 1 hour with a flux of 1×10^{12} neutrons/sec/cm² and measured after 10 minutes, 4,5 hours and one day respectively. The sample was then irradiated for 100 hours with the same flux and measured after 21 days. The following isotopes were found: Mn-56, Na-24, K-42, Ba-131, Ba-133, Ca-47, Cr-51, Sb-124, Zn-65 and Co-60.

4. Chloroplasts of spinach plants were also irradiated and analysed in order to know their mineral content. This information is important in relation to the interpretation of ion-uptake and release processes by these organelles.
5. To check the recovery of mercury under its various forms by different extraction techniques in soils, total mercury is determined by neutron activation analysis. This information is important in relation to the research on the behaviour of mercury and mercury compounds in soils. The sampling sites Valburg - a foreland soil from the river Rhine - and Hillegom - a soil in the bulb growing area - are chosen as experimental plots. Mercury is introduced into these soils respectively by the mercury polluted river water and as fungicide.
6. In collaboration with the Institute for Phytopathology at Wageningen a metal for labelling of onionflies - before release and after sterilization - had to be chosen. This labelling would permit detection of the sterilized flies, after capture, by neutron activation analysis. Experiments were done with gold, silver, bromium, copper, manganese, dysprosium and europium. Some of these elements, like silver and copper, appeared to poison the animal, the others were repelled by the chrysalis before changing into a fly. The experiments will be continued with iron, enriched with ⁵⁸Fe. Only one nuclide of iron (⁵⁸Fe with an abundance of 0.3 % !) can be activated, emitting a γ -radiation.

Short notes

- Changing of the irradiation position in the Barn-reactor core yielded a gain in flux from 7×10^{11} to $1,2 \times 10^{12}$ N/cm²/sec.
- The computer program has been adapted so that the terminal of a CDC 6600 computer can be used. The terminal is located at Wageningen, the computer at Rijswijk.

- Contractant de la Commission : Université Louis Pasteur de Strasbourg - Laboratoire de Biophysique des Rayonnements et de Méthodologie - 11, Rue Humann, Strasbourg.
 - N° du Contrat : SC 001-094-72-1 BIAN
 - Chef du groupe de recherche : R.RECHENMANN
 - Thème général du Contrat : Development of high-efficiency and high resolution ionographic methods - Applications to autoradiographic problems.
-

Les conséquences de l'introduction du concept de la sous-image corpusculaire, ainsi que des traitements dits "d'activation" de l'image latente, déjà décrits par ailleurs (1), conduisent à reconsidérer certaines des potentialités de l'émulsion nucléaire. L'exploration des nouvelles possibilités ainsi offertes est en cours, notamment dans le domaine de l'utilisation des émulsions à grains ultrafins, comme les différents types de détecteurs "Montréal".

Par ailleurs, les études de photographie fondamentale sont poursuivies, conjuguées avec certaines améliorations méthodologiques, en vue de l'obtention d'une efficacité et d'une résolution autoradiographique optimales.

Des résultats souvent contradictoires ont été obtenus par des auteurs traitant le problème de la résolution autoradiographique. Nous avons donc entrepris une étude sur la précision de localisation d'un isotope émetteur β situé dans un objet biologique. Dans un premier stade, une approche théorique de ce problème a été amorcée.

Les méthodes autoradiographiques "activées" sont appliquées à différentes études biologiques. Des résultats ont été obtenus dans le domaine de la différenciation cellulaire des tissus nerveux et de la survie d'organes impliquant l'exposition à des pressions très élevées (2 300 atm.).

Publications : R.RECHENMANN, E.WITTENDORP, High Efficiency Development Procedures for Nuclear Emulsions, J1. of Microscopy, Oct.1972, Vol 96, Pt.2, 227-244. M.KEDINGER, C.MENDEL, K.HAFFEN, E.WITTENDORP et J.F.GRENIER, Survival of chick embryonic organs submitted to high hydrostatic pressures. European J1. of Clinical and Biological Research (in press).

RESULTATS du PROJET N°1.

- Chef du projet et collaborateurs scientifiques :
R.RECHENMANN et E.WITTENDORP, C.BAIXERAS-AIGUABELLA.
 - Titre du projet : Development of high-efficiency and high-resolution ionographic methods - Applications to autoradiographic problems.
-

A. METHODOLOGIE.

Des essais de mise au point de révélateurs photographiques originaux ont été entrepris. Différentes approches ont été effectuées avec des solutions fortement solvantes ou non, organiques ou inorganiques, en vue de la mise en formule de révélateurs nouveaux, notamment de solutions réduisant les microcristaux en grains d'argent compacts. Certaines des formules que nous venons de mettre au point donnent des grains plus ou moins compacts, mais dont le diamètre moyen reste supérieur à celui des microcristaux d'origine, du moins pour un développement poussé.

Des expériences sont en cours en vue de l'amélioration de nos procédés d'intensification, entre autres par remplacement, dans les solutions d'activation, de l'or par d'autres métaux nobles, notamment le platine ou l'iridium.

Différents types de détecteurs ionographiques "Montréal" ont été soumis à nos procédés d'activation. Il en résulte que pour toutes les émulsions des types A,B,C,D,F et H, exposées à des sources de ^3H ou de ^{14}C , un accroissement du nombre de grains développés est obtenu par intensification à l'or. L'effet de l'activation est le plus élevé pour les émulsions à grains ultrafins.

B. RESOLUTION.

Le problème très complexe de la résolution autoradiographique a été traité par différents auteurs, les résultats obtenus étant en général peu concordants. Nous avons entrepris une détermination du pouvoir résolvant de l'autoradiographie au microscope électronique en nous basant sur un travail publié antérieurement (2). Dans un premier stade, nous avons amorcé une estimation théorique de la résolution dans le cas d'une source ponctuelle située dans deux configurations géométriques différentes par rapport aux microcristaux (Fig.1). Nous avons utilisé la définition et les principes de calcul décrits dans le travail précité, mais en introduisant le spectre d'énergie d'un isotope donné et en tenant compte de la rétrodiffusion des électrons. La réalisation d'un programme de calcul est en cours, qui permettra de déterminer la précision de localisation, en autoradiographie au microscope électronique et pour une combinaison donnée, des facteurs impliqués, comme la taille des

grains, le spectre du rayonnement émis, la géométrie de l'expérience, etc.

C. ETUDES BIOLOGIQUES.

I. Etude autoradiographique de la prolifération cellulaire de tissus nerveux.

En collaboration avec M. SENSENBRENNER^o et P. MANDEL^o, une étude de la multiplication éventuelle des cellules indifférenciées du cortex d'embryon de poulet et de leur évolution a été entreprise en appliquant des méthodes autoradiographiques spécifiques. Dans une première phase de nos travaux, nous avons appliqué la trace-histophotographie en vue de la détection de la thymidine marquée au ¹⁴C dans les cellules nerveuses isolées aux différents stades de leur évolution. On a choisi le ¹⁴C de préférence au ³H comme traceur pour diminuer l'influence du facteur selfabsorption des électrons émis au sein des tissus d'épaisseurs variables.

Les cellules isolées en culture ont été soumises à une coloration différentielle pour pouvoir discriminer les neurones des cellules gliales. Des recherches systématiques ont dû être entreprises pour trouver un colorant utilisable avant ou après les traitements autoradiographiques. Les préparations ont ensuite été soumises à un traitement autoradiographique activé

L'observation des autoradiogrammes montre que la plupart des cellules ont incorporé la thymidine ¹⁴C, ce qui confirme qu'elles étaient indifférenciées lors de la mise en culture. L'étude de l'influence des facteurs de différenciation présents dans un extrait de cerveaux embryonnaires, sur la multiplicité et la migration des gliales, est en cours.

II. Survie d'organes embryonnaires après l'action de très basses températures et de très hautes pressions.

En collaboration avec M. KEDINGER⁺, C. MENDEL⁺, K. HAFFEN⁺ et J. F. GRENIER⁺, nous avons appliqué nos méthodes autoradiographiques à des études sur la survie de l'intestin embryonnaire de poulet soumis à des hyperpressions. Les cultures de tissus traitées aux basses températures et aux très hautes pressions et les témoins avaient été marqués à la thymidine-tritiée avant de suivre les processus histologique et autoradiographique. L'observation des autoradiogrammes a mis en évidence une très forte incorporation de thymidine dans les noyaux (Fig. 2a et 2b), montrant que des pressions hydrostatiques allant jusqu'à 2 300 atm. peuvent être tolérées par ces organes.

Références : 1) RECHENMANN R. Proc. 7th Int. Conf. Corpusc. Phot. and Visual Solid Detectors, Barcelona 1970; N° EUR. 4688e, 1971. 2) RECHENMANN R., MELLONI M., WITTENDORP E. Acta Histochem. Suppl. 8, 139-154, 1967.

^o Centre de Neurochimie du C.N.R.S. - STRASBOURG

⁺ Unité de Recherches 61 de l'I.N.S.E.R.M. - STRASBOURG

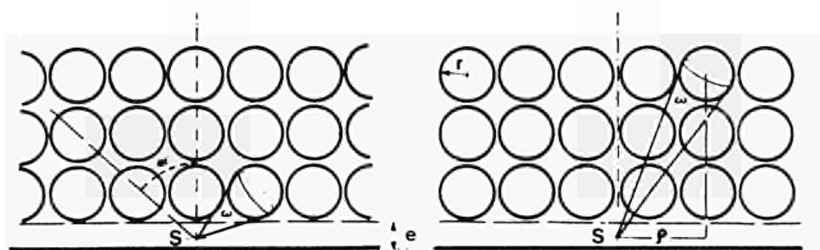
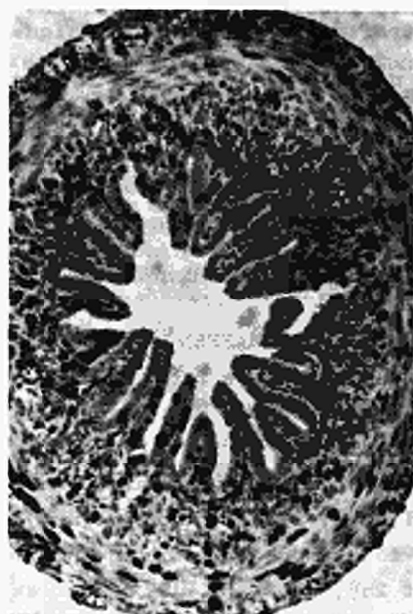
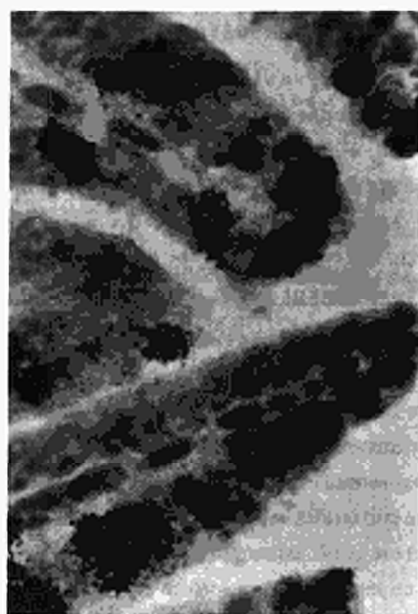


Fig. 1

Représentation schématique d'une coupe recouverte d'une épaisseur d'émulsion correspondant à 3 diamètres de grains (section transversale). Deux configurations géométriques de la source par rapport aux microcristaux sont considérés.



a



b

Fig. 2

Autoradiographies activées de coupes réalisées à partir de cultures d'intestins prélevés sur des embryons de poulet. Les tissus avaient été soumis à des hyperpressions de 2.300 Atm. avant marquage à la thymidine tritiée.

Emulsion: Ilford K5. Durée d'exposition: 18 heures. Développement à l'amidol après activation.

a) x 300_ b) x 1250

Contractant van de Commissie: Katholieke Universiteit
te Nijmegen (Nederland),
vertegenwoordigd door

dr. C.J.M. Aarts, directeur der Faculteit der Wiskunde
en Natuurwetenschappen.

Nr. SC 002 - 094 - 7 - 1 Bl. II

Hoofd van het researchteam: prof.dr. H.T. Linskens

Algemeen onderwerp van het contract: Radiation biochemistry
of styles and pollen of
incompatible plants.

Algemene omschrijving van de uitgevoerde werkzaamheden:

1. The effect of hot water treatment and x radiation on the oligo- and polysaccharides in styles of incompatible *Petunia* strains.
2. A new enzyme theory of incompatibility.
3. Influence of x-rays on the growth of pollen from *Pseudotsuga*.
4. RNA synthesis in styles of *Petunia* after pollination.

Resultaten van het project no. 1

Hoofd van het team en wetenschappelijke medewerkers:

prof.dr. H.F. Linskens

Titel van het project: Radiation biochemistry of styles and pollen of incompatible plants.

Beschrijving van de resultaten:

The project is part of the research activity of the Botanical Laboratory. The special aspects of the project were included in Spring 1972 after the appointment of drs. Kroes.

The results of the work during the second half of the year 1972 can be described as follows:

1. A new enzyme theory of incompatibility: H.W. Kroes

The basis of this theory is that pollentubegrowth inhibition is a consequence of lack of nutrient, instead of presence of an active inhibitor. An S1 haploid pollen tube lacks an enzyme (E1). In the diploid style (S1S2) the proteins P1 and P2 are synthesized. These proteins form a complex with a nutrient (sugar). In this way the nutrient only can be used for the pollentube-growth, if the protein-nutrient complex can be broken. For this a very specific enzyme is required, which is synthesized in the pollen tube in absence of the specific S-allele.

In the pollentube always the complete spectrum of these enzymes is synthesized, except that one for which the S-allele is present. A pollentube bearing S1 e.g. only produces enzymes E2.... Ex. In a diploid pollengrain in this way, always all enzymes are present, supposed that the S-allele independently are transcribed. This might explain why diploid pollen always gives a compatible growth.

The way this theory can be proved is by isolating the sugars and sugar-protein-complexes, and by correlating them to compatible or incompatible growth.

2. The effect of warm water treatment and α radiation on the oligo- and polysaccharide in styles of incompatible Petunia strains.

The experiments were designed to confirm some aspects of the new enzyme theory (see under 1.). Quantitative determination of the sugars was carried out by gas-chromatography in combination with thin-layer-chromatography. Both treatments increase the sugar content at low dosis (5000 r), at higher dosis (20,000 r) the reverse effect was observed. The investigations will be continued.

3. Influence of x-rays on the growth of pollen from Pseudotsuga

J.A.M. van der Donk (together with dr. G.K. Livingston, Seattle)

From experiments of Livingston it was known already that irradiation had an increasing effect on the growth-rate of this pollen. We tried to find out whether this phenomenon was a physical or physiological one. We therefore studied the RNA-synthesis in both cases (after irradiation at 64 kr and in the not irradiated control). The results revealed no difference between both groups. Presently, we are analyzing the rate of protein synthesis using polysomes extracted from irradiated and not irradiated pollen in a rat liver cell-free system.

4. RNA-Synthesis in styles of Petunia after pollination

In order to study the incompatibility reaction in Petunia, we study the RNA-synthesis in the style after pollination with both compatible and incompatible pollen, using ^{14}C -orotic acid as radioactive precursor. We found the highest RNA amount and the highest specific activity in selfed styles, the unpollinated style being the lowest.

Furthermore, a specific RNA (20S) was found after pollination, which was not present in unpollinated styles. It also was possible to correlate some low-molecular-weight RNA (6-8 S) and the S-cleaves present in the style.

Experiments are started to translate these RNAs in vitro and to analyze the proteins formed.

Lijst van publikaties in het kader van het contract:

- Deurenberg, J.J.M.: Influence of a virus infection on the self-incompatibility reaction. Inc. News Letter I, 9, 1972.
- van der Donk, J.A.W.M.: RNA-synthesis in styles of Petunia hybrida after self- and cross-pollination, compared to unpollinated styles. Inc. News Letter I, 10-12, 1972.
- Linskens, H.F. and Schrauwen, J.: Immuno-electrophoretic studies on pollen and style extracts from incompatible strains of Petunia hybrida. Inc. News Letter I, 16-17, 1972.
- Kroes, H.W.: An enzyme theory of self-incompatibility. Technical Report nr. 1, 1972.
- Kroes, H.W.: Oligo- en polysacchariden in stijlen van Petunia hybrida: het effect van warmwaterbehandeling en röntgenbestraling. Technical Report nr. 2, 1972.
- Schrauwen, J. and Linskens, H.F.: Ribonuclease in styles. Planta 102, 277-285, 1972.
- Linder, R. et Linskens, H.F.: Evolution des acides aminés dans le style d'Oenothera missouriensis vierge, autopollinisé et xénophyllinisé. TAG 42, 125-129, 1972.
- Linskens, H.F.: Die Hemm-Reaktion bei inkompatibler Bestäubung und ihre Überwindung. (russ.). in Fiziologia Rastenii 20 (1973) in press.

Administratieve gegevens

In het kader van het contract werd voor het tijdvak van 1 april 1972 tot en met 30 november 1972 aangesteld drs. H.W. Kroes.

Met ingang van 1 februari is in het kader van het contract in dienst genomen drs. L.W.J.W. Gilissen.

Sinds 1 oktober 1972 is drs. J. Schrauwen niet meer werkzaam voor de projecten in het kader van het contract. In plaats daarvan werden de werkzaamheden overgenomen door drs. J.A.W.M. van der Donk.

In het kader van het onderhavige project namen enkele doktoraalstudenten tijdens hun verblijf op het botanisch laboratorium aan het onderzoek deel.

During the year 1972 a visiting scientist from the USA, dr. G.K. Livingston, University of Seattle, was included into the program. He was granted by the Faculty of Science of the University of Nijmegen till August 31, 1972. He worked with pollen from forest material.

Als adviseur aan het project was verbonden de gasthoogleraar der faculteit der wiskunde en natuurwetenschappen prof.dr. Ernest Sondheimer (Syracuse University) van 1 januari 1972 tot en met 31 september 1972.

drs. J.J.M. Deurenberg en drs. J.A.W.M. van der Donk namen deel aan de Meeting of the Mutation Breeding Group in November 1972 te Casaccia (bij Rome). Zij hielden de volgende lezingen:

J.J.M. Deurenberg: *Petunia*: cytology, biochemistry and physiology.

J.A.W.M. van der Donk: Regulation of the incompatibility reaction in the *Petunia* system.

Prof. Linskens maakte deel uit van het International Scientific Advisory Committee (ISAC) van de associatie Euratom-Ital te Wageningen en nam aan de vergaderingen daarvan deel; hij hield zich speciaal met de projecten plant breeding, mutation breeding en incompatibility research bezig.

Contracting Research Institute: Department of Plant Breeding
Agricultural University,
Wageningen, the Netherlands

Number of contract: 094-72-1 BIAN s/c 003

Head of the research teams: Prof. Dr. Ir. J. Sneep

General subject of the contract: The use of mutations and mutation
techniques in mainly fundamental
plant breeding research.

General description of the project:

1. Mutation research in potato (van Harten, Bouter).

The work has been carried out in the way described under B. of this report. Research has gradually become more fundamental, i.e. concentrated on the effects of mutagenic treatment of shoot apices and the behaviour of histogenic layers after irradiation. Some experiments that are of more practical nature, are carried on yet.

2. a. Sporophytic Incompatibility in *Lobularia maritima* (Bos, Heemstra).
b. Gametophytic Incompatibility in *Solanum* spp. (Hermsen).

For practical reasons separate reports are attached for either incompatibility project.

3. Dwarf-rust in barley. (Parlevliet, van Ommeren).

In spite of the fact that the project on dwarf-rust in barley, an example of mutation breeding for disease resistance, will not be an Euratom project until 1974, an introductory paper is added, since the research started in 1972.

Results of the project: Potato (*Solanum tuberosum* L.) 1972

Leader of the team and co-worker: A.M. van Harten, H. Bouter

Title of the project: Mutation research in potato

1. Studies on chimeric structures.

1.1 Layer replacement in the potato apex is studied a.o. by trying to establish correlations between morphological - and histological observations.

A red-yellow splashed tubered monecto-chimera has been selected from the homohistic red tubered variety Désirée.

Single eyepieces of tubers were X-irradiated with 0, 750, 1500 and 3000 rad and partly planted in the greenhouse for morphological observations and partly used for histological investigations.

In rough lines regeneration of young sprouts of the 750 rad object mostly occurs from the highest axils. The 1500 rad object shows regrowth from lower situated axils. From the 3000 rad object regeneration begins in the lowest and least active axils of the sprouts.

Observations on adult tuberbearing plants provide, besides mutations and regeneration from permanent or temporarily damaged meristems, interesting results with regard to the red (perforations) and yellow (reduplications) tubers of the vm_1 plants.

Table I. Frequencies of colour changes to red, red-sectored and yellow coloured tubers in the vm_1 .

Objects	No of studied		Red or - sectored tubers in %		yellow tubers in %	
	plants	tubers	plants	tubers	plants	tubers
Control	65	353	25	7	0	0
750 rad	68	407	31	11	15	2
1500 rad	64	424	41	16	31	5
3000 rad	64	454	55	44	100	79

The results of the control, the occurrence of rare mutations from yellow to red, previous findings on 1085 vm_1

plants of this variety with comparable doses comprising 15% homohistic yellow tubered mutants and finally, the ample evidence that L_1 is genetically yellow highly corroborates the supposition of perforations and reduplications.

Histological observations have not been studied sufficiently yet to draw conclusions about the obtained high frequencies of solid red and yellow tubers.

- 1.2 Almost no adventitious bud formation has been observed in the 48,000 leaflets used up now in different trials performed in conditioned rooms, with applications of growth hormones and variations in environmental conditions. Experiments are continued because some objects showed + 10% of adventitious sprouts, but no differentiation of such sprouts occurred in an exact replica of these trials.
- 1.3 Screening suspected periclinal chimeras by means of testcrosses (information on L_2) and by adventitious sprouts from roots and de-eyed tuberslices (information on L_3) confirm information mentioned last year.

2. Experiments on disease resistances and yield capacity.

- 2.1 Rather good agreements have been found with former results concerning leafroll as well as Y virus. Trials will be continued to obtain lasting evidence.
- 2.2 Sub-clones of in 1969 re-irradiated Bintje material were included in last years investigations to study further increase of uniform resistance against Leafroll, Wart disease and Late Blight. Preliminary results show prospects for Leafroll, no advantage for Wart disease and Late Blight.
- 2.3 Comparative yield tests of promising sub-clones and their controls take a number of years, before a significant lasting increase is found. The 1971 results showed some sub-clones with significant increased yield capacity and correlation with former trials.

3. Generative programme.

- 3.1 Up to now none of the di-haploid ivyleafed clones reached flowering.

3.2 Many different test combinations and selfings were sown, which provide useful information about the inheritance of each of the four combined "marker" characters.

3.3 The new di-haploïd seedling 71A8 combining 4 independent marker genes unfortunately proved to be useless for mutation research by complete sterility, high sensitivity to irradiations and to low capacity of producing adventitious sprouts on de-eyed tubers and tuberparts.

Service, practical applications and courses.

Courses on mutation breeding (13 hrs) to University students and several (practical) lectures on this subject to participants of postgraduate courses and visitors. Guidance of graduate students in 3 research projects. Lecture about histological and morphological observations on an irradiated periclinal chimera of potato (Casaccia).

Publications and reports.

- HARTEN, A.M. van; H. BOUTER and A. van OMMEREN.
Preventing chimerism in potato (*Solanum tuberosum* L.).
Euphytica 21, 11-21 (1972).
- HARTEN, A.M. van; H. BOUTER.
Preliminary results of irradiated tubers of a di-haploïd Désirée clone. *Euphytica* 22 (1973).
- HARTEN, A.M. van; H. BOUTER and B. SCHUT (in press.).
Ivyleaf: Analysis of an induced leaf shape mutation in potato (*Solanum tuberosum* L. cv "Burmania").
- HARTEN, A.M. van; H. BOUTER.
Meeting of the mutation breeding contactgroup. Internal report I.T.A.L. Wageningen.

Results of the project: *Lobularia maritima* (L.) Desv.

Leader and co-worker: I. Bos, G. Heemstra.

Title of the project: The population genetics of the S-alleles of a sporophytic incompatibility system

1. Study of the relations of S-alleles.

HARUTA's method for determining the relation between 2 S-alleles is applied in its entirety to 9 S-heterozygotes. This method is based on reciprocal backcrossing of plants obtained through "forced" selfing and the appropriate parent. An advantage of the method is that at the same time S-homozygotes originate. However, *L. maritima* constitutes a problem, namely the impossibility to realize the selfed progenies by bud-pollination.

The average number of normal selfpollinations per S-heterozygote- necessary to obtain 1 seed-ranged from 6 to 521. The germination of these laboriously obtained seeds was poor (5-27 %) if no dormancy was allowed, but increased (to 46 - 71 %) after a dormancy of 2½ months. The number of plants per I₁-line ranged therefore from a minimum of 2 to a maximum of 46. Subsequently, each I₁ plant was at least 50 times crossed reciprocally with his parent. Based on the results of these crosses the relations within pairs of S-alleles have been determined and the S-homozygotes discovered. Identification of the S-homozygotes will follow by means of a diallel cross of the homozygotes.

2. Equilibrium frequencies of S-heterozygotes.

A theoretical study of the equilibrium frequencies of S-genotypes in a population with only 3 S-alleles has been completed, based on a mating system with zygote elimination. As a result of this composition of the population in equilibrium has been found for all possible 185 combinations of a series of S-allele relations for stigma phenotype and one for pollen phenotype.

The combination 20 supposes, for stigma as well as pollen, the S-allele relations: S_a>S_b, S_a<S_c and S_b>S_c. This gives the equilibrium frequencies:

S-genotype frequencies						S-allele frequencies		
S _a S _a	S _b S _b	S _c S _c	S _a S _b	S _a S _c	S _b S _c	S _a	S _b	S _c
.0787	.0787	.0787	.2546	.2546	.2546	.3333	.3333	.3333

It is feared that the significance of the applied mating system has been overestimated in the literature. A mating system with pollen elimination, on the contrary, gives an adequate description of the natural situation.

Incidental mutation of an allele, e.g. $S_a \rightarrow S_a'$, may give rise to a new gene. Unlike for the gametophytic incompatibility system, an a priori prediction of how a mutant allele is absorbed is impossible for the sporophytic system.

Extending the study to a system with 4 S-alleles is therefore a logical continuation.

Leader: Dr. Ir. J.G.Th.Hermesen.

Title of the project: Origin and inheritance of self-compatibility in induced dihaploids of Solanum tuberosum L.

The incompatibility system in the genus Solanum is gametophytic and generally monogenic. This implies that doubling the number of chromosomes of a self-incompatible diploid genotype brings about self-compatibility, whereas induction of haploid parthenogenesis in an autotetraploid genotype gives rise to self-incompatible dihaploid plants. Notwithstanding this rule a number of self-compatible dihaploids were obtained from *S. tuberosum*, cv. Gineke. An investigation of the cause and inheritance of this self-compatibility was started, in order to elucidate what kind of natural mutations in Gineke or its ancestors have taken place, leading to overcoming the incompatibility barrier. The information thus obtained might show the way in which such result may be reached most efficiently through artificial mutagenic treatment of cultivars.

Apart from selfing the self-compatible dihaploids (coded G16 and G254, reciprocal crosses with a self-incompatible dihaploid (G609) from the same cultivar, and reciprocal backcrosses of the F₁-hybrids with the parental dihaploids were carried out. All populations were tested for self-(in)compatibility and complete diallels were made in order to determine the incompatibility- and compatibility genotypes.

The results obtained hitherto are briefly as follows. Self progenies of self-compatible G16 and G254 consist of self-compatible plants only, being reciprocally compatible with their respective parent. The results from reciprocal F₁'s and backcrosses are summarized in the tables 1 and 2 respectively.

Table 1. Ratios self-incompatible (si): self-compatible (sc) in the F₁'s and results from intra-F₁ matings. The incompatibility- and compatibility-groups are given by indices (si₁, si₂; sc₁, sc₂). Equal indices point to a certain correspondence of genotypes, which is clear from table 2. G16 belongs to group sc₃, G254 to sc₁, G609 to si₅.

Reciprocal crosses	F ₁ -ratios si : sc	Intra - F ₁ matings			
		complete diallels		sc ♀ x si ♂	si ♀ x sc ♂
		sc $\overline{\times}$ sc	si $\overline{\times}$ si		
G254 x G16	3 : 26	all comp	si ₂ , si ₃	sc ₁ , sc ₂ , sc ₃	all comp.
G16 x G254	7 : 22	do	si ₁ , si ₂	sc ₁ , sc ₂ , sc ₃	do
G254 x G609	21 : 17	do	si ₄ , si ₅	sc ₄ , sc ₅	do
G609 x G254	28 : 10	do	si ₁ , si ₄	sc ₁ , sc ₄	do
G16 x G609	17 : 20	do	si ₁ , si ₂ , si ₄ , si ₆	sc ₁ , sc ₂ , sc ₄ , sc ₆	do
G609 x G16	21 : 14	do	si ₁ , si ₂ , si ₄ , si ₆	sc ₁ , sc ₂ , sc ₄ , sc ₆	do

Conclusions from table 1: G16 and G254 as well as G254 and G609 have one S-allele in common at the S-locus (two si-groups). G16 and G609 have no common S-allele (four si-groups). All crosses involving a sc male parent are compatible independent of the female genotype (column 3 and 6). In crosses with a sc parent as a male, certation in the pollen causes a surplus of si F₁-plants (explanation below).

Table 2. Results from reciprocal crosses between all si- and sc-groups and the three parental dihaploids (+ = compatible, - = incompatible). S₁' is an interchromosomal duplication of S₁ with a mutated stylyr regulatory cistron.

F ₁ -groups		G254		G16		G609	
		sc ₁ (S ₁ S ₃ /S ₁ ')		sc ₃ (S ₃ S ₄ /S ₁ ')		si ₅ (S ₁ S ₂)	
code	genotype	♀	♂	♀	♂	♀	♂
si ₁	S ₁ S ₃	-	+	+	+	+	+
si ₂	S ₁ S ₄	+	+	+	+	+	+
si ₃	S ₃ S ₄	+	+	-	+	+	+
si ₄	S ₂ S ₃	+	+	+	+	+	+
si ₅	S ₁ S ₂	+	+	+	+	-	-
si ₆	S ₂ S ₄	+	+	+	+	+	+
sc ₁	S ₁ S ₃ /S ₁ '	+	+	+	+	+	+
sc ₂	S ₁ S ₄ /S ₁ '	+	+	+	+	+	+
sc ₃	S ₃ S ₄ /S ₁ '	+	+	+	+	+	+
sc ₄	S ₂ S ₃ /S ₁ '	+	+	+	+	+	+
sc ₅	S ₁ S ₂ /S ₁ '	+	+	+	+	+	-
sc ₆	S ₂ S ₄ /S ₁ '	+	+	+	+	+	+

Hypothesis to explain the results. An interchromosomal duplication of S_1 through mutual weakening of different S -alleles in the pollen, brings about self-compatibility and also cross-compatibility, if the male parent is self-compatible. On the other hand the duplicated S_1 allele is inactive in the style (stylar regulatory cistron mutated, hence the symbol S'_1), which may be concluded from the compatibility of the crosses $S_2S_3/S'_1 \times S_1S_2\delta$ and $S_2S_4/S'_1 \times S_1S_2\delta$. Pollen carrying S'_1 shows a certative disadvantage. The duplicated segment seems to be incorporated in a whole chromosome no centric fragments being found.

Conclusion: in cv. Gineke or more probably in its ancestors an interchromosomal duplication of the S_1 -allele has taken place, followed by a mutation which inactivated the stylar regulatory cistron. Then cv. Gineke may tentatively be given the genotype $S_1S_2S_3S_4/S'_1$. Therefore 50% of its dihaploids are expected to be self-compatible.

Publications in 1972.

M.M.F.Abdalla and J.G.Th.Hermsen, 1972. Unilateral incompatibility: hypotheses, debate and its implications for plant breeding.

Euphytica 21: 32-47.

M.M.F.Abdalla and J.G.Th.Hermsen, 1972. Plasmons and male sterility types in *Solanum verrucosum* and its interspecific hybrid derivatives.

Euphytica 21: 209-220.

M.M.F.Abdalla and J.G.Th.Hermsen, 1972. Diploid parthenogenesis and androgenesis in diploid *Solanum*. Euphytica 21: 426-431.

Introduction to the project: Dwarfrust in barley.

Leader of the team and co-worker: J.E. Parlevliet, A. v. Ommeren.

Title of the project: Mutation breeding for disease resistance.

The history of resistance breeding leaves no doubt as to the general usefulness to agriculture of major resistance genes. However, the history has also shown that the benefits conferred by major genes, are more often than not, short-term benefits. There is also no good reason for thinking that major genes obtained by induced mutation should last any longer. This has led to an increased interest in other types of resistance, especially those based on poly genes. It is hoped that this will provide a more stable resistance because of the following reasoning. When we change the genetic composition of the host, the pathogen tends to restore the lost equilibrium by changing too, if possible. This is experienced as the breaking down of the acquired resistance. In order to regain its old position after an increase of resistance in the host the pathogen has to absorb new virulence genes matching the new resistance genes into its gene pool. The gene pool has a delicately balanced structure, and its components are, in Dobzhansky's term, coadapted. Hence a replacement of alleles at one locus is bound to produce in its wake a displacement of allelic frequencies at other loci. This secondary effect may in turn generate further changes at the original locus until either the old balance is regained (genetic homeostasis) or a new one achieved (Lerner, 1958).

It is easily visualised, that the process of absorbing genes into the gene pool is easier the smaller the number of genes to be absorbed and the more neutral their effect is on the fitness of the pathogen population as a whole. In most cases of major genic resistance the gene pool of the pathogen has to absorb only one virulence gene at a time and these virulence genes are often, especially in resistances of the hypersensitivity type (very often used) not appreciably associated with fitness. This is a most favourable situation for the pathogen to readapt to the resistant variety without too much strain on the coadapted state of the gene pool. If, on the other hand, many genes, not neutral with respect to fitness, have to be absorbed into the pathogen's gene pool in order to erode introduced resistance it is quite possible that no new balance within the gene pool can be found. The gene pool resists taking up these virulence genes in order not to lose its

coadapted state. The resistance appears to be stable. It is assumed that polygenically based resistances of the quantitative type belong to this group.

To widen the field of mutation breeding for disease resistance it seems necessary to study the possibilities of inducing polygenically based, quantitative disease resistance. That this is possible is indicated by the work of SIMONS (1971). He was able to induce mutations for tolerance to crown rust (Puccinia coronata) in oats. Tolerance to crown-rust in oats seems polygenically inherited and is a complex character, compounded of real tolerance and of resistance of the quantitative type.

Literature.

- LERNER, I.M., 1958. The genetic basis of selection. John Wiley & Sons, New York.
- PERSON, C. and SIDHU, G., 1971. Mutation Breeding for Disease resistance. Int. Atomic Energy Agency, Vienna 1971. 31-38.
- SIMONS, M.D., 1971. Phytopathology 61, 1064-1067.

Associato della Commissione: Comitato Nazionale per
l'Energia Nucleare, Laboratorio Agricoltura
N° del contratto: 094-72-1 BIAN a/c 004
Capo del grupo di ricerca: Prof. A. Bozzini
Tema generale del contratto: FUNDAMENTAL AND APPLIED
RESEARCH WITH THE GAMETOPHYTIC SYSTEM OF SELF-INCOMPATIBILITY IN HIGHER PLANTS

The researches carried out at the Casaccia in the field of incompatibility dealt, in 1972, with the following aspects:

-identification of the chromosome bearing the S-locus in Nicotiana glauca and analysis of the cytological events leading to the establishment of self-compatibility (negative mutations).

-study of the mechanisms responsible for the generation of new S-alleles (constructive mutations) in Lycopersicon peruvianum and in Antirrhinum majus.

These investigations are closely related and complementary to those performed at the Association EURATOM-ITAL by A.J.G. van GASTEL and G. Bredemeyer (see report by these scientists) and the entire research programme is carried out as a single joint effort in Wageningen and at the Casaccia. In addition, and always with the aim of improving our understanding of the breeding system of allogamous species and of the mechanisms which lead to the rejection of incompatible pollen tubes, an electron microscopic analysis was performed, at the University of Siena (Prof. G. SARFATTI, Dr A. CRISTINI, Dr M. PACINI), of self-incompatibility and unilateral interspecific incompatibility reactions in vivo.

Risultati del progetto N° 1: see report of the Association
EURATOM-ITAL

Risultati del progetto N° 2

Capo del progetto e collaboratori scientifici:

D. de Nettancourt (Casaccia), A.J.G. van Gastel (Wageningen)

B. Donini (Casaccia), M. Devreux (Casaccia), G. Bredemeyer.

Titolo del progetto: The spectrum of spontaneous and induced mutations at the S-locus: a comparative analysis on the origin and nature of constructive (generation of new alleles) and negative (genetic losses) mutations

1) Analysis of mutation spectra at the S-locus of N. alata: see report of the Association EURATOM-ITAL

2) The detection and origin of spontaneous mutations at the S-locus of inbred plants of L. peruvianum.

In order to elucidate the mechanisms operating when inbred plants of L. peruvianum spontaneously generate new S-alleles (de Nettancourt et al, Theor. Appl. Genet. 1971), the same S_1 and S_2 alleles which had been studied previously in one inbred genetic environment were placed, after a complete diallel analysis, test-crosses and identity tests, in several different genetic backgrounds for ascertaining the influence of such backgrounds on the frequency and specificity of spontaneous constructive mutations at the S locus. As the S-genotype of the staminate parent which was used for introducing modifications in the genetic background is known ($S_4 S_5$), the same material will permit the detection of an eventual relation between mutation specificity and the identity of the S-alleles which have been associated, one generation earlier, to S_1 and S_2 . In 1972, the work involved the conduction of a diallel test (20 plants) in the progeny of $S_1 S_2 \times S_4 S_5$ crosses,

the determination of each of the 4 genotypic classes present in the progeny and the production, by means of crosses between S_1S_4 or S_1S_5 and S_2S_4 or S_2S_5 individuals, of S_1S_2 genotypes in new genetic backgrounds. Such S_1S_2 plants will be submitted in 1973 to obligate inbreeding, detection tests and ancestry tests.

A very similar analysis has been initiated this year on the progenies of the inbred self-compatible mutant for which cytological analyses recently demonstrated (de Nettancourt et al, in press) that the self-compatibility character was due to the fact that the plant was tetraploid and produced heteroallelic pollen. The progenies of this tetraploid are interesting from the point of view of constructive mutations because a number of fertile tetraploid plants are clearly self-incompatible and because a number of self-compatible tetraploids appear to be cross-incompatible with one another. Diallel tests are presently conducted between such plants with the aim to find out if certain tetraploid genotypes really have the property of building up a specific rejection mechanism which displays the precise features of the incompatibility system and eventually results, as in the inbred diploids, from the generation of new S-alleles or from the reactivation of a previously inert locus.

3) The analysis of spontaneous and induced S-mutations in Antirrhinum (responsible scientist: Prof. B. DONINI)

The necessary material for initiating this part of the project has been introduced in 1972 at the Casaccia (self-incompatible accessions of A. siculum, A. orontium and A. latifolium). In the self-compatible species A. majus a very interesting mutant has been detected which either displays female sterility or represents an unique case of a change from self-compatibility towards stylar self-incompatibility. Test-crosses are underway to find out which of these two explanations is the correct one.

Risultati del progetto N° 3

Capo del progetto e collaboratori scientifici:

D.de Nettancourt (Casaccia), A.J.G. van Gestel (Wageningen)

F. Carluccio (Casaccia).

Titolo del progetto: Establishment of linkage relationships with the S-locus of self-incompatible plants and identification of the S-bearing chromosome.

This problem, which has been tackled, in 1972, at both the cytological (Casaccia) and genetical (Wageningen) point of view, is in many respects complementary to the biochemical approach followed by G. Bredemeyer (project N° 4) for the early detection of S-genotypes.

1) the cytological identification of the S-bearing chromosome in *N.alata* and analysis of the mechanism leading to the formation of self-compatibility mutations with a centric fragment.

The discovery has been made in 1972 that silar part mutants of *N.alata* and the entire clonal populations from which they were derived are unstable at the S-locus and display a duplication of the satellite region in one of the two chromosome 3, the other chromosome 3 and the 8 remaining pairs of chromosomes being apparently normal and identical to those of the standard stocks of *N.alata*. In some instances, somatic cells could be observed where the normal and the modified chromosome 3 had lined up one after the other and had fused their satellites to form a complex with a clear central constriction. In a few cases, the complex appeared to liberate itself and to evolve into a free centric centric fragment very similar to the fragments which characterize negative pollen part S-mutations in *N. alata* (Pandey, 1968). Such a finding not only suggests that the chromosome 3 of *N. alata* is involved in the determination of the self-incompatibility phenomenon but

also ~~indicates~~ a participation of the nucleolar organizing region in the control of the incompatibility mechanism. Attempts will be made in 1973, with the help of Prof. D'AMATO at Pisa, to find out if ribosomal cistrons are located on the duplicated segment of the modified chromosome 3 and on the centric fragments which are so typical of pollen-part mutants in N. alata.

2) the establishment of linkage relationships with the S-locus of N. alata: see report of the Association EURATOM-ITAL.

Complement to project N° 2 and project N° 3.

It is obvious that the various research goals which are defined within the general theme of the programme can hardly be reached unless a clear knowledge is available of the basic processes which govern incompatibility relationships in higher plants. Whereas the biochemical aspects of the problem are being studied in details within the framework of the Association (see report by G. Bredemeyer and from the University of Nijmegen), it had not yet been possible to define and to initiate researches on the fine structure of incompatibility reactions. It has been our achievement in 1972 to build with Prof. Sariatti, Dr Cresti and Dr Pacini, at the university of Siena, a small cooperation programme which was entirely restricted to an electron microscopic analysis of incompatibility in the genus Lycopersicum and which has yielded a considerable amount of basic information. The observations performed and the results obtained to date are described, together with the names of the scientists involved, in the following sections of the present report:

1) Ultrastructural aspects of the self-incompatibility mechanism in Lycopersicum peruvianum (D. de Nettancourt, M. Devreux, A. Bozzini, M. Cresti, A. Pacini, G. Sariatti).

The experimental results obtained indicated that the tip

of the incompatible pollen tube bursts open after that, the outer-wall having considerably expanded in the intercellular spaces of the conducting tissue, the inner-wall has disappeared and numerous particles have accumulated in the tube cytoplasm. These particles, which measure approximately 2,000 Å in diameter and give a negative reaction to the test of Thiéry, differ in many respects from the vesicles normally present in compatible pollen tubes growing through the style; they appear to resemble, in some cases, the spheres which are discharged by the compatible pollen tubes having reached the embryo-sac.

It is considered that these observations support the current belief on the tube wall as the site of action for the incompatibility proteins and suggest that self-incompatibility is not a passive process resulting from a lack of growth stimulation but an active event which leads to the destruction of the incompatible pollen tubes. The degradation mechanism involved appears similar to the one which enables the compatible pollen tube to release its content in the degenerated synergid and presents some analogies with the lytic process taking place in virus-infected cells. The general hypothesis is presented that the particles observed in the cytoplasm of self-incompatible pollen tubes consist of a mixture of incompatibility proteins and of basic constituents of the tube-wall. All these results and conclusions have been published this year (J. Cell Science, 1972).

2) The ultrastructure of interspecific unilateral incompatibility in the genus *Lycopersicum*. (D. de Nettancourt, M. Devreux, U. Laneri, A. Pacini, M. Cresti, G. Sarfatti).

Always at the Laboratory of Electron Microscopy of the University of Siena (Prof. G. Sarfatti), observations have been made of the ultrastructure of the pollen tubes present in the styles of *Lycopersicum esculentum* (cultivated tomato) and *L. peruvianum* after reciprocal crosses between the two species. Such an analysis of the unilateral incompatibility barrier

which isolates the two species when L.peruvianum is used as the pistillate parent rendered possible a comparison between the processes involved and those which had been previously analysed in the case of self-incompatibility in L.peruvianum. This confrontation, which was also carried out by means of fluorescence techniques, has permitted to find out that for both types of incompatibility the rejection process is characterized by a progressive disappearance of the callose rich inner-wall of the pollen tube and by an accumulation of bi-partite particles in the tube cytoplasm. In the case of unilateral incompatibility, however, the elimination of the inner-wall was restricted to the very tip of the tube and was accompanied by a dismantelling of the outer-wall which had never been observed in self-incompatible tubes. As a result of this complete degradation of the apical wall, the cross-incompatible pollen tube merely opens in the stylar tissue and does not accomplish the bursting process which had been found so typical of the self-incompatibility reaction. These observations (Caryologia 1973, in press) support the hypothesis that unilateral incompatibility is governed by a mechanism which is related but not identical to the one controlling self-incompatibility.

Since self-incompatibility systems are operating in more than 60,000 species of higher plants and since interspecific incompatibility is a major obstacle to the improvement of many cultivated crops, it is considered that the results presented in these last two paragraphs do not only complement the various projects included in the present research theme but are also of direct relevance to many other fields of agronomic interest.

Risultati del progetto N° 4: see report of the Association
EURATOM-ITAL

P U B L I C A T I O N S

(progetti N° 2 & N°3)

1) Published in 1972

D. de NETTANCOURT . Self-incompatibility in basic and applied researches with higher plants. Genet. Agrar. 26:163-216 (1972)

D.de NETTANCOURT,M.DEVREUX,A.BOZZINI,M.CRESTI,E.PACINI and G. SARFATTI. Ultrastructural aspects of the self-incompatibility mechanism in Lycopersicum peruvianum Mill. J. Cell Science (1972).

F.CARLUCCIO, A.J.G. van GASTEL and D. de NETTANCOURT. Modifications of chromosome 3 in a leaf-propagated S_2S_3 clone of Nicotiana alata. Inc. News.,1:6-7 (1972)

B.DONINI. Female-sterility or stylar incompatibility in Antirrhinum majus . Inc. News.,1:10 (1972).

G. van GASTEL. Spontaneous stylar-part mutations at the S-locus in Nicotiana alata Link and Otto. Inc. News.,1:12-13 (1972).

A.J.G. van GASTEL, P.DIJKHUIS,D.deNETTANCOURT and F.CARLUCCIO. The inheritance of marker mutations obtained by means of the leaf-propagation technique in Nicotiana alata. Inc. News,1:14 (1972).

2) in press (1973)

D. de NETTANCOURT,M.DEVREUX,U.LANERI,E.PACINI,M.CRESTI and G.SARFATTI. Ultrastructural aspects of unilateral interspecific incompatibility between Lycopersicum peruvianum and L.esculentum . Caryologia (1973).

F. CARLUCCIO, D.de NETTANCOURT and A.J.G. van GASTEL. On a possible involvement of chromosome 3 in the formation of self-compatibility mutations in N. alata. Proceed. FAO/IAEA/EUCARPIA symp. ,Bari (1972).

D. de NETTANCOURT,F.SACCARDO,U.LANERI,E.CAPACCIO,M.WESTERHOF, R.ECOCHARD. Self-compatibility in a spontaneous tetraploid of Lycopersicum peruvianum . Proceed. FAO/IAEA/EUCARPIA symp.,Bari (1972).

Vertragspartner der Kommission: Professor Dr. Gottschalk
Institut für Genetik
der Universität Bonn
53 B o n n
Kirschallee 1

Nr. des Vertrags: 094-72-1 BIAN s/c 007
Leiter der Forschungsgruppe: Professor Dr. Gottschalk
Allgemeines Thema des Vertrags:

"Genphysiologische und biochemische Untersuchungen an strahleninduzierten Mutanten von Pisum"

Darstellung der durchgeführten Arbeiten

Es wurden 6 röntgeninduzierte Mutanten mit züchterisch brauchbaren Merkmalen sowie 18 Rekombinanten, die nach Kreuzung verschiedener Mutanten selektiert worden waren, im Hinblick auf ihre Samenproteine analysiert und mit der Ausgangsform verglichen. Hierbei wurden folgende Kriterien berücksichtigt:

- der Gesamtproteingehalt der Samen,
- das Verhältnis der Globuline und Albumine,
- die Aminosäure-Zusammensetzung der Proteine.

Außerdem wurden einige dieser Mutanten und Rekombinanten an der Universität von Udaipur in Indien angebaut, um ihre Leistungsfähigkeit unter subtropischen Bedingungen zu testen. Die in Deutschland und Indien durchgeführten Parallelversuche zeigten für einige Genotypen ein unterschiedliches Anpassungsvermögen an Hitze und Trockenheit. Nach Vermehrung dieses Materials in Udaipur werden uns vergleichende Proteinanalysen der in Deutschland und Indien aufgezogenen Genotypen Aufschluß über die Abhängigkeit der Synthese von Samenproteinen von bestimmten Klimafaktoren geben.

Ergebnisse des Projekts Nr. 1

Leiter: Professor Dr. Gottschalk
Mitarbeiter: Privatdozent Dr. Müller
Titel: Die quantitative und qualitative Analyse der Samenproteine von Mutanten und Rekombinanten in Abhängigkeit von der Samenproduktion

Der Proteingehalt unserer Ausgangsform ist mit 19% des Trockengewichts der Samen innerhalb des Weltsortiments relativ niedrig. Eine unserer verbänderten Mutanten zeigt eine Steigerung von 24% gegenüber der Stammform. Sie ist zwar kleinsamig, produziert jedoch aufgrund der Stengelverbänderung sehr große Samenmengen je Pflanze. Wenn man die Samenproduktion der Mutante mit dem Proteingehalt in Beziehung setzt, so liegt die Proteinproduktion der Mutante im dreijährigen Mittel etwa 40% über derjenigen der Stammform (Abb. 1). Fütterungsversuche an Ratten, die von Dr. Eggum in Dänemark durchgeführt wurden, zeigten, daß die Samenproteine der Mutante in ernährungsphysiologischen Beziehungen den Proteinen der Stammform entsprechen.

Als weiteres Kriterium für die Qualität der Proteine ist in Abb. 2 für einige Mutanten sowie ihre Rekombinanten der Gesamtgehalt der ernährungsphysiologisch entscheidenden essentiellen Aminosäuren dargestellt. Hierbei hat sich die Kombination der beiden mutierten Gene 68 (Erhöhung der Anzahl der Samenanlagen im Fruchtknoten) und 46 (Frühreife) als besonders günstig erwiesen. Rekombinanten, die für beide Gene homozygot sind, besitzen 25% mehr essentielle Aminosäuren als die Stammform.

Die quantitativen Veränderungen in der Aminosäure-Zusammensetzung der Samenproteine sind auf genetisch bedingte Verschiebungen ihrer beiden Hauptfraktionen - der Globuline und Albumine - zurückzuführen.

Ergebnisse des Projekts Nr. 2

Leiter: Professor Dr. Gottschalk
Mitarbeiter: Dr. Milutinović
Titel: Die Samen- und Proteinproduktion von Positiv-
Mutanten in Abhängigkeit von Klimabedingungen

Überraschenderweise kann die ertragreichste Mutante unseres Sortiments - die bereits erwähnte verbänderte Form - für die Erbsenzüchtung in Indien nicht verwendet werden, da sie unter den dortigen Klimabedingungen nicht blüht. Eine zweite, ebenfalls verbänderte Mutante hingegen (251A) erreicht die Samenproduktion der Stammform, während sie in Deutschland erheblich über den Kontrollwerten liegt. Eine unserer Rekombinanten (R 177A) dürfte wegen ihrer hohen Samenproduktion für die indische Pflanzenzüchtung von Interesse sein, da etwa 25% des gesamten Proteinbedarfs der indischen Bevölkerung aus Samenproteinen von Leguminosen gedeckt werden. Die frühblühende Rekombinante R 46C liegt im Samenertrag zwar beträchtlich unter den Vergleichswerten der Stammform, zeigt jedoch eine gewisse Toleranz gegenüber Pilz- und Virus-Erkrankungen. Außerdem kann sie wegen ihres vorverlegten Blüh- und Reifetermins noch in Trockengebieten Indiens mit unzureichender Bewässerung kultiviert werden, die für andere Genotypen ungeeignet sind. Einzelheiten sind aus Abb. 3 ersichtlich. Die Befunde zeigen, daß die geprüften Genotypen gegenüber bestimmten Klimafaktoren ganz andere Anpassungsontima haben als die Stammform.

Veröffentlichungsliste

Folgende Arbeiten wurden im Rahmen der beiden Forschungsprojekte im Jahre 1972 veröffentlicht bzw. zum Druck gegeben:

- Gottschalk, W. Combination of mutant genes as an additional tool in plant breeding. Induced Mutations and Plant Improvement; IAEA Vienna, 199-218 (1972)
- , - The penetrance behaviour of a Pisum gene causing stem-bifurcation. Pisum Newsletter 4, 5-6 (1972)
- , - Die Kombination mutierter Gene. Biol.Zblatt 91, 91-109 (1972)
- , - Harmonische und disharmonische Genkombinationen in der Mutationszüchtung. Z.Pflanzenzüchtung 67, 221-232 (1972)
- Gottschalk, W. and S. Kumar. The response of pea mutants to moderate and semi-tropical conditions. Z.Pflanzenzüchtung 67, 95-102 (1972)
- Gottschalk, W. und V. Milutinović. Untersuchungen zur Heterosis bei Selbstbefruchtern I, II. Genetika (Beograd) 1972; im Druck
- Gottschalk, W. and H. Müller. The protein yield of some Pisum mutants and recombinants. Pisum Newsletter 4, 7-8 (1972)
- Müller, H.P. The genetic control of quantitative composition of seed proteins in mutants and recombinants of Pisum sativum. Genetika; im Druck
- Müller, H.P. and S.R. Baquar. Genetically controlled variation in seed protein of some recombinants of X-ray induced Pisum mutants. Angew. Bot.; im Druck
- Müller, H.P. and W. Gottschalk. The reaction of pea mutants to different light intensities. Pisum Newsletter 4, 43 (1972)

-,-

The influence of different climatic factors upon the growth of pea mutants. Pisum Newsletter 4, 41-42 (1972)

-,-

The quantitative and qualitative situation of the seed proteins in mutants and recombinants of Pisum sativum. Proc.Meet. on the Use of Nuclear Techniques for the Improvement of Seed Protein; IAEA Vienna 1972; im Druck

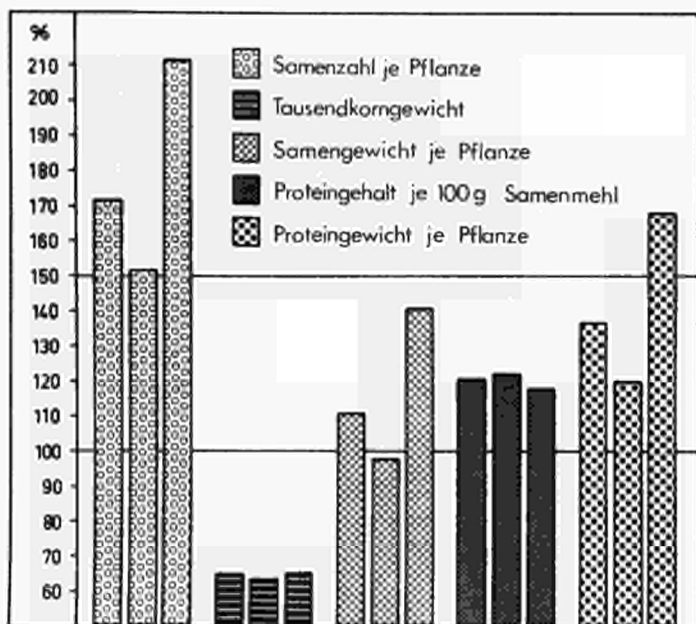


Abb. 1: Die Beziehungen zwischen Samen- und Proteintrag bei der verbänderten Erbsen-Mutante 489C in drei aufeinanderfolgenden Jahren, bezogen auf die Vergleichswerte der Ausgangsform = 100%. Trotz der Kleinsamigkeit liegt die Proteinproduktion der Mutante weit über den Kontrollwerten.

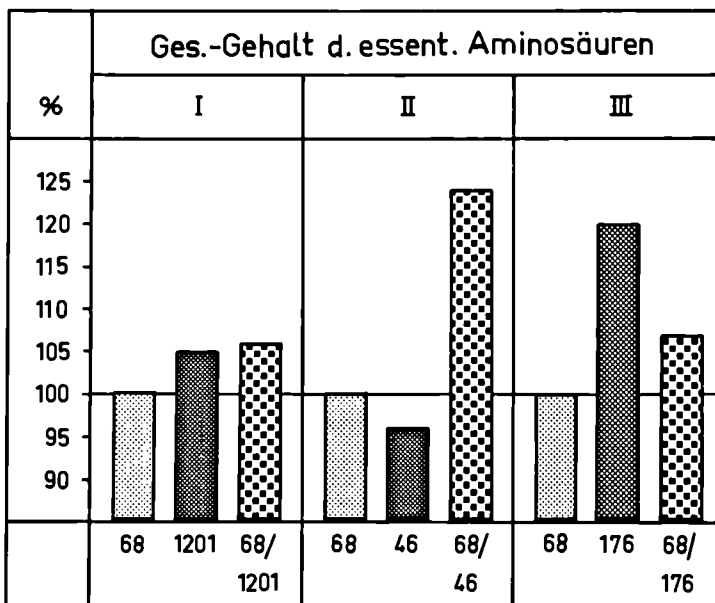


Abb. 2: Die Gesamtmenge der essentiellen Aminosäuren von 4 Erbsen-Mutanten und 3 Rekombinanten im Vergleich zur Stammform.

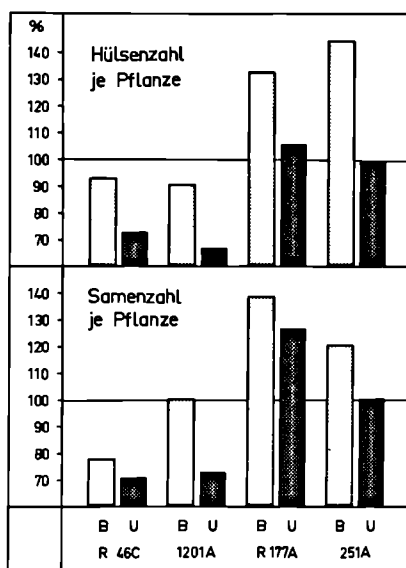


Abb. 3: Vergleich der Samenproduktion einiger Genotypen unter den klimatischen Bedingungen von Bonn/Deutschland (B) und Udaipur/Indien (U), bezogen auf die Vergleichswerte der Ausgangsform.

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Nummer des Vertrages: SC 08/94-72-1 BIAN

Leiter der Forschungsgruppe:

Prof. Dr. H. Gaul, Leiter der Abteilung Pflanzengenetik

Allgemeines Thema des Vertrages:

Production and selection of barleys with vertical and horizontal resistance against mildew (Erysiphe graminis)

Under project 1 we shall establish a collection of mildew resistant barleys through the induction of mutations. It will consist of plants with vertical as well as horizontal resistance. For the secondly mentioned resistance type an effective selection method has to be developed.

In addition to the already existing mildew resistant mutants new ones will be produced by chemical and physical mutagens. The studies will result in information on the frequency of resistant mutants. Another aim is to obtain information on the efficiency of mutant production.

Under project 2 genetic analyses of each mildew resistant mutant are carried out. The mode of inheritance and the number of resistance genes are studied. Selected mutants will be used to demonstrate the independent variation of pleiotropic characters and the separation of mildew resistance from undesired features, like reduced grain yield, sterility and low tillering.

Ergebnisse des Projekts Nr. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Dr. J. Grunewaldt
Prof. Dr. H. Gaul

Titel des Projekts:

Selection of mildew resistant barley mutants following mutation induction

Darstellung der Ergebnisse:

In 1962 our collection of mutagen-induced mildew resistant barleys was initiated. It was increased year by year and comprises now 91 mutants.

Out of these we selected 48 strains which are resistant to the mildew population of the Cologne area that is extremely aggressive. Presumably the race C₅ is included in this population. In 1972 field observations of the 48 strains indicated also resistance at our new location Grünbach, close to Munich.

Another 43 barley mutants were subjected to the natural infection in the field at Grünbach. In greenhouse tests, conducted in previous years, it was shown that these mutants are resistant to race C₂, kept in Weihenstephan.

In 1972 a program was started to induce new mildew resistant mutants in order to enlarge the number of genes and alleles involved in our collection. Varieties or strains will be treated which possess no resistance but which have other desirable characters, like strawstiffness, high protein content of seeds and earliness.

In 1972 three varieties were treated with X-rays and methyl methane sulfonate. The first selection will take place in M₂-generation. Tests for vertical and, if available, for horizontal resistance will follow.

Ergebnisse des Projekts Nr. 2

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Dr. J. Grunewaldt
Prof. Dr. H. Gaul
Dipl.-Landwirt V. Lind

Titel des Projekts:

Influence of the genetic background on the expression of
mildew resistance

Darstellung der Ergebnisse:

All mildew resistant mutants in spring barley included in our collection will be analysed genetically.

In 1972 we backcrossed 24 mutants with their mother varieties to find out the mode of inheritance of mildew resistance. The mutants were induced in "Haisa II", "Gerda" and "Matura" which are varieties of Central Europe.

In addition, we crossed the mutants each with another to study whether or not they are allelic. Reciprocal crosses were not carried out. From each mutant we pollinated the flowers of two spikes. We obtained seeds from 105 cross combinations. The F_1 - and F_2 -generations will show if the mildew resistance is caused by the same or by different alleles and genes respectively. The crossing experiments will also give information about the dominance and/or recessivity of the genes for resistance.

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Nummer des Vertrages: SC 08/94-72-1 BIAN

Leiter der Forschungsgruppe:

Prof. Dr. H. Gaul, Leiter der Abteilung Pflanzengenetik

Allgemeines Thema des Vertrages:

Production and selection of barleys with increased protein quantity and improved protein quality

The genetical improvement of protein quantity and protein composition in small grains is performed by mutation breeding methods. Our general object is the combination of high protein content, improved protein quality and high kernel yield in selected mutants.

The basis for a successful selection is a broad genetical variability in both protein characters, and useful analytical methods for their determination.

The research project for protein-improved grain varieties thus consists of four consecutive steps.

The first step deals with analytical problems of the protein determination and their biometrical implications for selection.

In a second step we consider the variability of mutants, and the genetical relation of protein with characters like yield, resistance and other morphological and physiological features.

The third step includes the influence of environmental components like climatic conditions, soil, fertilization and other technical factors on the genotypical variation of improved protein characters.

In the final step the selected strains will be exploited for breeding purposes.

At present we are engaged with investigations of step 1 and step 2.

Ergebnisse des Projekts Nr. 1

Leiter des Projektes und wissenschaftliche Mitarbeiter:

Prof. Dr. H. Gaul
Dipl.-Landwirt V. Lind

Titel des Projekts:

Utilization of natural and mutagen-induced protein variability
in diploid barley

Darstellung der Ergebnisse:

In order to exploit the potential of genetic variability, we developed from different commercial barley varieties mutant spectra; these were classified into groups of macro- and micro-mutations. Besides these two groups of diploid mutants we tested tetraploid strains as a third group of mutants. All mutants originated from treatments with EMS, X-rays, and Gamma-rays of a cobalt-60 source.

The protein content was determined with the Kjeldahl-method and was measured in % crude protein in dry matter. The biological value of barley is limited by the essential aminoacid lysine. We decided to measure the lysine content with " μg lysine per 100 mg dry matter". For chemical analysis a method similar to the analysis proposed by CYMMIT in Mexico is used. This method is based on a colorimetric evaluation of a specific dye-lysine complex. For the preceding hydrolysis we used the enzyme pronase.

First results of diploid and tetraploid barley mutants showed strong negative correlations between % crude protein in dry matter and % lysine in crude protein, with an average of $r = -0.9$. The relation of "% protein in dry matter" to "% lysine in dry matter" however is positive with $r = 0.6$. In simultaneous selection for both protein characters it seems more favourable, therefore, to use protein and lysine measures on the basis of absolute amounts in dry matter.

Another problem is the reliability of analytical measurements. The conformity of replicated determinations within each sample

was tested by correlations between three replications. Higher coefficients for the protein analysis clearly indicate that the Kjeldahl method is more reliable.

One of the problems of breeding procedures is to find an efficient selection rate in subsequent generations. Since the selection rate is directly correlated with the selection intensity and thus with the genetic advance of selection, we applied different selection rates in experiments with different phenotypical variability. In five experiments a total selection rate of 15 % applied to 526 strains resulted in an increase of protein from 13.3 % to 14.3 %, on the average. With the same selection rate the lysine content was raised from 0.58 % to 0.60 %. The advance of selection mainly depends on the variation of a character. The protein content with higher genetic variability is therefore thought to lead much faster to improved strains than the lysine content. Mutagenic treatment which results in a broad genetical variation is therefore of primary interest. In these experiments a superiority of either EMS or X-ray treatment could not be inferred.

Other investigations concentrated on the extent of the variation potential of mutants. Since both protein characters are simultaneously under selection, we use a two-dimensional distribution for the evaluation of improved mutants. Explanation and results of this diagram are given under project 2.

Ergebnisse des Projekts Nr. 2

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof. Dr. H. Gaul
Dipl.-Landwirt W. Friedt

Titel des Projekts:

Utilization of the protein variability of diploidized tetraploid barley

Darstellung der Ergebnisse:

A comparison of 66 autotetraploid barley strains with 305 micro-mutants and 165 macro-mutants resulted in information on the variation potential of both protein characters. Figure 1 shows the extreme variation line for all three mutation groups. To enable a comparison of the mutants with their initial varieties, the mean values of the distribution areas and of the initial varieties are presented.

As can be seen, the three mutant groups differ considerably. Macro- and micro-mutants exhibit an overall variation in % crude protein from 9 to 16 % and for lysine from 470 to 650 μg . In contrast to diploid mutants, the tetraploid strains range from 14.5 to 18 % in crude protein and from 540 to 720 μg in lysine. The mean values of the distribution areas reflect these results best. They show at the same time that the average of the corresponding mutants surpass the values of the initial varieties. The variability among artificially produced mutants seems to be not less than is known from natural variation.

For selection and subsequent cross-breeding those mutants are chosen which surpass the mean value of the group in both characters. Therefore some of the tetraploid strains can be expected to give raise to improved lines as well as some of the micro- and macro-mutants. About 25 % of all strains, obtained after irradiation or chemical treatment, are superior to their initial varieties in protein and lysine.

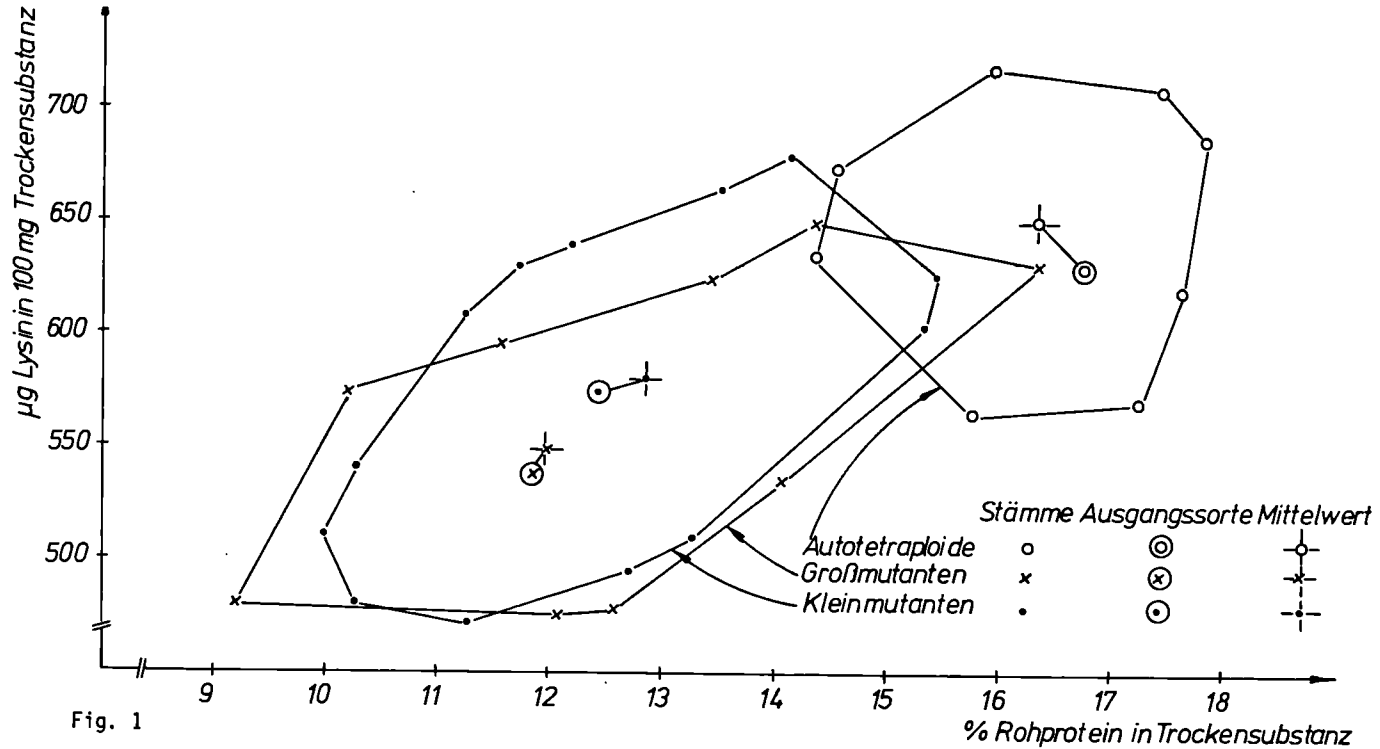


Fig. 1

Diagram of the field of variation for protein- and lysine content in barley. Results of 165 macro-mutants, 305 micro-mutants and 66 autotetraploid strains. The graph shows the extreme variation lines only, the mean values of the three mutant groups and the values of the initial varieties from which the mutant strains are derived.

Publications 1972:

Gaul, H., Ulonska, E., Lind, V., Walther, H.:
Studies of selection for high protein and lysine content in
barley mutants.
Proc. Symp. IAEA/FAO, Neuherberg (Munich), 1972 (in press)

Lind, V., Gaul, H.:
Mutagen-induced mildew resistance in barley.
Report, Meeting of the Mutation Breeding Contact Group,
Wageningen, Association EURATOM-ITAL, Cassaccia, 1972 (in press)

Ulonska, E., Gaul, H., Baumer, M., Fritz, A.:
Breeding on protein quantity and quality in barley.
Proc. Symp. IAEA/FAO, Neuherberg (Munich), 1972 (in press)

Walther, H., Gaul, H.:
Variation of protein quantity and quality in barley.
Report, Meeting of the Mutation Breeding Contact Group,
Wageningen, Association EURATOM-ITAL, Cassaccia, 1972 (in press)

Vertragspartner der Kommission: Gesellschaft für Strahlen-
und Umweltforschung mbH. München, 8042 Neuherberg/München.

Nr. des Vertrages: SC 09/94 - 72 - 1 BJAN

Leiter der Forschungsgruppen: Priv. Doz. Dr. W. Kühn

Allgemeines Thema des Vertrags:

Strahlenanalyse im Landbau

Im Vordergrund standen im Berichtszeitraum folgende Untersuchungen:

1. Feuchtigkeitsaustausch zwischen Atmosphäre und oberen Bodenschichten unter Berücksichtigung von Temperaturinversionen im Wüstenklima (Negev). Durch Markierung des Wasserdampfes mit Tritium wird untersucht, inwieweit die periodisch auftretende Inversionen im Hinblick auf die Wasserversorgung von Nutzpflanzen die Kultivierung von Wüstengegenden ermöglichen. Diese Arbeiten werden in Zusammenarbeit mit dem Botanischen Institut der Hebrew University of Jerusalem (Forschungsstelle Avdat, Negev) ausgeführt. Die Arbeiten im Jahre 1972 betrafen insbesondere die Simulation der Temperaturinversionen in einem Bodenmodell.

2. Entwicklung eines Meßverfahrens zur Bestimmung des Gesamtfrischgewichtes von Pflanzen an ganzen Beständen in verschiedenen Wachstumsperioden. Dieses Vorhaben wurde von Pflanzenzüchtern angeregt, für die eine zerstörungsfreie und schnell Massenbestimmung in gewissen Stadien des Wachstums von außerordentlichem Nutzen ist.

Zu diesem Zweck sind im Berichtszeitraum die theoretischen und technischen Voraussetzungen eines geeigneten Scanningverfahrens erarbeitet worden.

3. Entwicklung und Prüfung eines röntgendiagnostischen Verfahrens zur Feststellung von Rotfäule in lebenden Bäumen. Frühere Untersuchungen an ausgewählten Objekten haben gezeigt, daß es möglich ist, durch Röntgenaufnahmen und durch gleichzeitig damit verbundene Absorptionsmessungen den inneren Zustand eines Baumes zu beschreiben. Auf diese Weise können im Rahmen der Landschaftspflege wertvolle Bäume erhalten werden.

Für den praktischen Einsatz wurde im Jahre 1972 eine transportable Röntgenanlage erstellt. Im Zuge der Abholzung einer 250-jährigen Lindenallee mit mehr oder weniger geschädigten Bäumen konnte mit dieser Anlage das Verfahren auf seine Nützlichkeit geprüft werden. Gesunde und kranke Bäume wurden dabei selektiert.

4. Die Indikator-Aktivierungsmethode hat sich bei vielen Untersuchungen als nützlich erwiesen, weil anstelle von Radionukliden vielfach aktivierbare Tracer Verwendung finden können. Eine zusammenfassende Darstellung der Grundlagen des Verfahrens soll 1973 publiziert werden.

5. Die Entwicklung eines Verfahrens zur Feuchtigkeitsmessung in dünnen Lößbodenschichten (Proj. Nr. 4) wurde abgeschlossen. Die praktische Erprobung des Gerätes soll 1973 in der Negev-Wüste in Israel erfolgen.

Veröffentlichungen

1. W. Kühn, H.P. Schätzler:
"Mass Determination of Plantations by Absorption of Gamma-Radiation"
Paper presented at the III. Annual Meeting of the European Society of Nuclear Methods in Agriculture. Sept. 26 - 29, 1972, Budapest.
2. W. Kühn, M. Elmdust, M. Evenari:
Bestimmung der Evaporation des Wasserdampfes im ariden Lößboden während der nächtlichen Temperaturinversionsphase durch Tritium-Markierung bestimmter Bodenschichten.
Kerntechnik, 14. Jahrg. (1972) Nr. 2
3. W. Dammann, M. Elmdust, W. Kühn:
Kontinuierliche Bestimmung des Wasserdampfes im Boden durch Tritiumaktivierung.
Kerntechnik, im Druck
4. W. Kühn:
"Strahlenanalyse bei landwirtschaftlichen Untersuchungen und im Landbau"
Grundlagen der Landtechnik, Bd. 22 (1972) Nr. 2

Administrative Bemerkungen

Durch den Wechsel eines wissenschaftlichen Mitarbeiters wurde anstelle von Herrn Dipl.Landwirt M. Elmdust Herr Dipl.Physiker C. Bunnenberg mit der Bearbeitung des Projektes Nr. 3 beauftragt.

Da das Projekt Nr. 4 bezüglich der direkten Feuchtigkeitsmessung keine weiteren Laborarbeiten mehr erforderlich macht, sind umfangreiche theoretische und experimentelle Arbeiten des Projektes Nr. 1 von Herrn Dipl.Physiker H.P. Schätzler mitübernommen worden.

Das Projekt Nr. 4 ist insofern noch erweiterungsfähig, als mit der Einrichtung voraussichtlich auch das Kapillarpotential des Bodens meßbar ist. Eine dahingehende Erweiterung des Projektes Nr. 4 würden wir im Hinblick darauf anstreben, wenn die Bedürfnisse der Praxis dies wünschenswert erscheinen lassen.

Ergebnisse des Projekts Nr. 1..

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Priv.-Doz. Dr. W. Kühn Dipl.-Phys. H. P. Schätzler

Titel des Projekts :

Bestimmung der Pflanzenmassen an Beständen

Im Vordergrund stehen Frischgewichtsbestimmungen an Pflanzen und Pflanzenbeständen mit Hilfe eines Scanning-Verfahrens, sowie Feuchtigkeitsmessungen in dünnen Bodenschichten. Im Jahre 1972 wurden für das obige Projekt umfangreiche theoretische und technische Grundlagen erarbeitet und anlässlich der III. ESNA Tagung im September 1972 in Budapest vorgetragen. Sie beinhalten ein Scanning-Verfahren mit der die Masse eines Getreidefeldes berührungslos bestimmt werden kann. Die mit diesem Meßverfahren verbundenen Probleme, wie die Bestimmung der Masse aus den gemessenen Flächengewichten, die Wahl geeigneter Strahlungsgeometrien und damit verbundene Koinzidenzanordnungen, die die im exponentiellen Schwächungsgesetz nicht einbezogene Streustrahlung elektronisch unterdrücken soll, das räumliche Auflösungsvermögen bei der Massenbestimmung, statistische Probleme bei diskontinuierlichen Scannen und die Optimierung der Meßgenauigkeit mit Hilfe geeigneter γ -Strahler konnten theoretisch geklärt werden. Dabei hängt die Energie der Strahlung im wesentlichen vom Flächengewicht und damit von der Größe des Feldes ab. Bei einer durchstrahlten Strecke von 4 Meter ergeben je nach Fehlerursache der Anordnung (statistischer oder instrumenteller Fehler überwiegt) und je nach dem Wassergehalt der Pflanzen Am-241 (60 keV) oder Co-57 (122 keV) maximales Auflösungsvermögen des Flächengewichtes.

Ferner wurde ein mathematisches Modell zur getrennten Bestimmung der Halm- und Ährenmasse aus der Gesamtmasse und anderer charakteristischer Pflanzengrößen abgeleitet, und der technische Aufbau der Maßeinrichtung projektiert. Zur Bestätigung der theoretischen Überlegungen in Laborversuchen wurde außerdem das Phantom eines Getreidefeldes, bestehend aus Getreide äquivalenten und ähnlich geformten Plexiglasstäben, ausgearbeitet und zum Teil fertiggestellt. Außerdem wurde unter Abwägung der physikalischen und technischen Gesichtspunkte und der praktischen möglichst problemlosen Anwendung der Methode die elektronische Meßapparatur konzipiert. Aus dem Vergleich verschiedenster Detektoren und Meßgeräte hat sich ein Szintillationszähler mit einem im Multiscalingbetrieb arbeitenden kleinen Vielkanalanalysator, auch hinsichtlich der später notwendigen elektronischen Datenverarbeitung der Meßwerte, als am geeignetsten erwiesen.

Die Entwicklung eines Verfahrens zur Feuchtigkeitsmessung in dünnen Lößbodenschichten wurde abgeschlossen. Die praktische Erprobung des Gerätes soll 1973 in der Negev-Wüste in Israel erfolgen.

Ergebnisse des Projekts Nr. 4.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Dipl.-Phys. J. Handl

Titel des Projekts :

Untersuchungen zur Diagnose und Therapie von Holzfäule in
lebenden Bäumen

Es wurde die Möglichkeit geprüft, mit Hilfe von Röntgenaufnahmen Krankheiten, insbesondere Rotfäule, in lebenden Bäumen sichtbar zu machen. Zu diesem Zweck wurden Durchleuchtungen an sichtlich kranken Holzscheiben ausgeführt, die bei ungefilterten Röntgenenergien um 75 KeV recht scharfe Leuchtschirmbilder vom kranken Bereich lieferten. Bei diesen Aufnahmen treten die durch Holzfäule befallenen Stammportien im Verhältnis zum gesunden Holz in Form von Linien deutlich hervor, so daß sie als solche gut gegenüber nicht befallenen Bereichen röntgenologisch erkennbar sind. Eine Selektion der kranken Bäume ist daher prinzipiell möglich.

Im Hinblick auf die praktische Anwendung des Verfahrens war festzustellen, inwieweit sich ein bestimmter Fehler im Innern bei unterschiedlichen Durchmessern gerade noch nachweisen läßt. Hierfür wurde ein Phantom einer Baumscheibe benutzt, dessen Durchmesser sich verändern ließ und bei dem innere Bereiche beliebig symmetrisch oder asymmetrisch aushöhlbar sind. Durch Röntgenaufnahmen wurde die Abhängigkeit der photometrisch erhaltenen Schwärzungsdifferenzen zwischen massivem Holz und diesen künstlich erzeugten Fehlern vom Baumdurchmesser festgestellt. Aufgrund dieser Messung der Fehlererkennbarkeit ergab sich, daß eine Flächengewichtsänderung im Holz von ca. 2% bei einem Durchmesser von 50 cm gerade noch nachweisbar ist.

Die Filme wurden, um Einflüsse des Baumdurchmessers auszuschließen, mit einer vorgewählten Dosis bestrahlt, so daß sich für unterschiedliche Durchmesser verschiedene Zeiten bis zur gleichen Dosis ergeben. Durch diese zusätzliche Absorptionsmessung wurden weitere Aussagen über den inneren Zustand eines Baumes gewonnen.

Um die bisherigen Ergebnisse in der Praxis prüfen zu können, wurden 15 250-jährige Lindenbäume vor ihrem Fällen mit einer im Berichtszeitraum erstellten fahrbaren Röntgenanlage untersucht. Eindeutig konnten mit Hilfe des Verfahrens die tatsächlich kranken von den gesunden Bäumen unterschieden werden.

Die Befunde wurden nach dem Fällen der Bäume von danach aus den Stämmen herausgeschnittenen Scheiben optisch bestätigt.

Ergebnisse des Projekts Nr. 3.

Leiter des Projekts und wissenschaftliche Mitarbeiter :

Dipl.-Phys. C. Bunnenberg

Titel des Projekts :

Messung von Evaporation und Transpiration in Böden

Im Berichtszeitraum 1972 wurde eine Anlage erstellt, mit der Einflußgrößen und Randeffekte untersucht werden können, die bei der Simulation der in der Negev-Wüste registrierten Temperaturinversionen im Boden auftreten können. Zur Nachahmung der Inversionen wird die Atmosphäre in dieser Versuchsanlage über einer Bodensäule beheizt bzw. gekühlt, während die Temperatur in 50 cm Bodentiefe konstant gehalten wird.

Bei diesem Modell eines nicht unendlich ausgedehnten, sondern begrenzten Bodenprofils sind zwei Einflußgrößen besonders zu berücksichtigen:

1. der horizontale Wärmetransport durch die Mantelflächen der Bodensäule und
2. der Wasserdampftransport an den Berührungsflächen von Bodensäule und Gefäßwandung

Gegenstand eingehender Untersuchungen war der horizontale Wärmetransport in der Bodensäule. Die Messungen geben Aufschluß darüber, welche Wärmeisiermaßnahmen nötig sind und welche Abmessungen die Bodensäule haben muß, damit der horizontale Wärmetransport gegenüber dem vertikalen vernachlässigbar klein und dadurch die erforderliche unendliche Geometrie in guter Näherung erreicht wird. Dabei ist zu berücksichtigen, daß mit steigendem Bodenvolumen der energetische und apparative Aufwand stark zunimmt. Aus dieser Sicht und unter Berücksichtigung des Tritiumbedarfs soll die unendliche Geometrie in vertretbaren Grenzen gehalten werden.

Außerdem liefern die Untersuchungen Anhaltspunkte über die Temperaturregelung in der Bodensäule und Aussagen über die Anforderungen, die an eine geplante automatische Steuerung der Temperaturinversionen im Tag-Nacht-Zyklus gestellt werden müssen.

Zur Bestimmung der Randeffekte beim Wasserdampftransport an den Grenzflächen von Boden und Gefäß wurde eine Vorrichtung gebaut, die es gestattet, den aus verschiedenen Zonen der Bodenoberfläche aus-

tretenden tritiummarkierten Wasserdampf getrennt zu messen. Die Ergebnisse dieser Messungen sind sowohl für eine sinnvolle Dimensionierung der Bodensäule als auch für die Bestimmung eines für den Feuchtigkeitstransport in Frage kommendes Volumen wichtig, wenn dieses frei von Randeffekten sein soll.

Diese Untersuchungen dienen außerdem der Erprobung eines Verfahrens, das es erlaubt, die Tritiummarkierung zu einem bestimmten Zeitpunkt im periodisch verlaufenden Inversionszyklus zu applizieren.

Teil B Ergebnisse des Projekts Nr. 4

Leiter des Projekts: Dipl. -Phys. H. P. Schätzler

Titel des Projekts: Feuchtemessung in dünnen Bodenschichten durch Absorption von Gammastrahlung in einem kapillar porösen Körper.

Im Berichtszeitraum wurde zur zusätzlichen Bestimmung der Bodendichte, die bei der Feuchtigkeitsmessung über das Kapillarpotential in die Messung eingeht, eine zweite Dichtesonde entwickelt. Es wird dabei anstelle von Cs-137 eine niederenergetische Strahlenquelle, bestehend aus Am-241 benutzt. Dadurch konnte das räumliche Auflösungsvermögen und die Meßempfindlichkeit der Dichtemessung verbessert werden. Die Folge hiervon ist eine höhere Genauigkeit der Feuchtigkeitsmessung. Für die Praxis wurden Eichkurven unter Berücksichtigung unterschiedlicher Bodendichten und Feuchtigkeiten aufgenommen. Dabei ist das Auswerteverfahren den praktischen Bedingungen angepaßt worden.

Die eigentlichen Versuche im Laboratorium waren damit abgeschlossen. Im Rahmen des

"International Training Course On Basic Principles
Of Isotopes And Radiation Equipment Used In Soils
Research"

der IAEA und des Institut für Biophysik der Universität Hannover vom 19. 7. - 6. 9. 1972 wurde die Meßeinrichtung von Bodenwissenschaftlern aus 20 Ländern zur Messung von Feuchte und Dichte an Lößboden benutzt.

Die für das Jahr 1972 vorgesehenen praktischen Versuche im Negev wurden in Übereinstimmung mit Prof. M. Evenary von Dep. of Botany der Hebrew University Jerusalem auf 1973 verschoben.

Da mit kapillar porösen Körpern primär das Kapillarpotential des Bodens gemessen wird, in das Feuchtigkeit und Dichte letztlich mit eingehen, kann das Verfahren auch zur Bestimmung des Kapillarpotentials herangezogen werden. Es wird deshalb daran gedacht, die Methode dahingehend zu erweitern.

ZELLKULTUREN

CELL CULTURE

CULTURE DE CELLULES

Commission Associate: Istituto di Genetica della Università, Pisa

Contract Number: 106-72-1 BIOI

Head of the research team: Prof. F. D'Amato

Co-workers: Drs. M. Buiatti, A. Bennici, C. Geri, P.G. Cionini

Subject of the contract: Cytology and genetics of plant tissues
and cells grown in vitro.

Report on activities 1972

During 1972, the following investigations have been carried out:

1) Cell dedifferentiation in plant tissues grown "in vitro".

Work on the cytochemistry and biochemistry of Nicotiana glauca pith tissue grown in vitro has been continued. In situ RNA-DNA hybridization of dedifferentiating N. glauca pith cells and DNA binding with tritiated actinomycin D both showed labeling in the nucleolar region and in the cytoplasm after 48-70 hours from the primary explant (NUTI RONCHI, BENNICI and MARTINI).

Extraction of tritium-labeled DNA from the pith tissue showed maximum nuclear labeling 75 hours after the primary explant; labeling decreased afterwards. CsCl gradient analytical ultracentrifugation revealed the existence of a DNA satellite with a density (1.720 g/cm^3) higher than that of main band DNA (1.694). This satellite was clear at 48 hours of culture, but disappeared later on. All these data suggest a process of transient gene amplification (probably of ribosomal cistrons) and extrusion of the amplified DNA into the cytoplasm, during the initial phases of dedifferentiation in Nicotiana glauca pith tissue (BUIATTI, DURANTE, GERI, GIORGI and PARENTI).

2) Molecular biology of the embryo suspensor of Phaseolus coccineus.

The polytene chromosomes of the embryo suspensor of Phaseolus

coccineus were used to localize the cistrons coding for ribosomal RNA (rRNA). Tritiated rRNA was prepared from hypocotyls of P.coccineus fed in the dark with ³H-uridine and used for in situ hybridization. It was found that the ribosomal cistrons (rDNA) are localized in the nucleolus organizing system (satellite, nucleolar constriction and organizer) of the satellited chromosome pairs I(S₁) and V(S₂), in the proximal heterochromatic segment of the long arm of chromosome pair I and in the terminal heterochromatic segment of the chromosome pair II. The micronucleoli which are produced by the satellite and organizer of chromosome pair I also contain ribosomal cistrons. It has also been shown that DNA sequences other than rDNA are amplified in the polytene chromosome cells of P. coccineus (AVANZI, DURANTE, CIONINI and D'AMATO 1972). Binding of tritiated actinomycin D to DNA in the polytene chromosomes of P. coccineus has given indication that the chromosomal regions bearing ribosomal cistrons are rich in G-C (CIONINI and AVANZI 1972).

At present, work is in progress on the cytological localization of the cistrons for 5S rRNA in the polytene chromosomes of P. coccineus (AVANZI, DURANTE, CIONINI and D'AMATO).

After the demonstration that the embryo suspensor of P. coccineus is an outstanding material for molecular biology, it has been decided to start work in vitro on this system. In October 1972, isolated suspensors and embryos with suspensors have been brought in vitro in two different media designed to i) give normal development in vitro of embryos and suspensors and ii) induce callus formation from both embryo and suspensor (BENNICI and D'AMATO).

3) Genetics of growth and differentiation "in vitro" of cauliflower.

The relation of genotype to growth and differentiation in vitro has been studied using 6 lines of cauliflower deriving from 4 years of inbreeding. High heritability values have been obtained for callus growth, number of callus-forming petals and number of

initiated roots. Data were also obtained on the hormonal plasticity of the lines using two concentrations of auxins and cytokinins. Striking differences between lines were observed (BARONCELLI, BUIATTI and BENNICI 1972).

4) "In vitro" culture of protoplasts.

Recently BENNICI and PAGLIAI (1972) developed a technique for the isolation of protoplasts (cells deprived of their pecto-cellulosic wall by enzymatic digestion) in high concentration in a nutrient medium. Using this technique, work has been continued on the growth and behaviour in vitro of protoplasts of the tumorous hybrid Nicotiana glauca x N. langsdorffii and its parental species (BENNICI).

Publications

(For the results of papers marked by an asterisk, see Report on activities 1971).

- 1 Avanzi S., Durante M., Cionini P.G. and D'Amato F., 1972. Cytological localization of ribosomal cistrons in polytene chromosomes of Phaseolus coccineus. Chromosoma, 39:191-203.
- 2 Baroncelli S., Buiatti M. and Bennici A., 1972. Genetics of growth and differentiation in vitro of Brassica oleracea var. botrytis. I: Differences between inbred lines. Zeitschrift für Pflanzenzüchtung (in the press).
- 3* Bennici A., Buiatti M., Tognoni F., Rosellini D. and Giorgi L., 1972. Habituation in Nicotiana bigelovii tissue cultures: different behaviour of two varieties. Plant and Cell Physiology, 13:1-6.
- 4* Bennici A. and Pagliai M., 1972. Protoplasts from tissues grown in vitro of the tumorous hybrid Nicotiana glauca x Nicotiana langsdorffii. Informatore Botanico Italiano, 4:72.
- 5 Cionini P.G. and Avanzi S., 1972. Patterns of binding of tritiated actinomycin D to Phaseolus coccineus polytene chromosomes. Experimental Cell Research, 75:154-158.

6*Durante M.,Parenti R.,Buiatti M. and Geri C.,1972. Electrophoretic patterns of RNases in normal and habituated plant tissues. Informatore Botanico Italiano, 4:76.

7*Durante M.,Parenti R.,Buiatti M. and Geri C.,1972. Plant tissue DNA disc-electrophoresis on agarose-acrylamide gels. Informatore Botanico Italiano, 4:76.

Associato della Commissione: Comitato Nazionale
per l'Energia Nucleare, Lab.Agricoltura
N° del contratto : 107-72-1 BIOD

Capo del gruppo di ricerca: Prof.A.Bozzini

Tema generale del contratto: Applications of the
in vitro cultures to radiobiological researches
and mutagenesis of higher plants

The researches carried out in 1972 were
principally oriented in three directions:

- 1) The use of in vitro culture techniques
in relation to self-incompatibility problems.
- 2) The research at the macromolecular level
of the modifications induced by in vitro culture in
different plant tissues.
- 3) Genetical studies on isogenic lines of
tobacco derived from anther cultures and mutagenic
treatments of the microspores from a diploid iso-
genic line.

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici:

M. Devreux, D. de Nettancourt, U. Laneri.

Titolo del progetto: The use of in vitro culture techniques in relation with self-incompatibility problems.

1.1. Anther cultures of self-incompatible species were performed with diploid and tetraploid plants of Nicotiana glauca and Lycopersicon peruvianum. This research was carried out to obtain haploids and thereafter isogenic lines of self-incompatible species which should permit the study of the influence of isogeny on the eventual spontaneous generation of new alleles at the S-locus. All the culture with N. glauca failed (giving only embryoids but no plantlets) while, following the technique recently described by Gresshoff and Doy (Planta, 1972), we have obtained with L. peruvianum numerous callus proliferations from anthers. These calli were transplanted on a second medium to ensure the proliferation of the calli and, afterwards on a third medium to try to induce the differentiation of plantlets. We are now observing the first root formations.

1.2. We have pursued the in vitro cultures of tissues coming from different parts of self-incompatible plants with the aim of realizing a "bank of tissues" characterized by known S-alleles. We have now good techniques to obtain callus proliferation from petals of N. glauca and from styles and stem disks of L. peruvianum.

A good culture medium (De Langhe, comm.pers.1972) was also utilized with success to induce plantlet regeneration from calli of L.peruvianum. We also regenerated autotetraploids of L.peruvianum and these plants were, as expected, self-compatible. With different genotypes, we were able to maintain callus proliferation and to regenerate plantlets. With one particular genotype, we regenerated many plantlets for finding out in the near future if in vitro cultures can also induce mutations at the S-locus or elsewhere on other genes. First attempts were carried out to identify the specific isoenzymes of the peroxidases in callus tissues coming from well known S genotypes.

1.3. With regard to the biological screening method for mutated pollen at the S-locus, the in vitro culture of N.alata style pollinated with incompatible and compatible pollens shows that it is very easy to observe in the medium the growth of compatible pollen tubes. The conditions necessary for preventing the growth of all incompatible tubes in the style are now defined. Next year, first attempts with irradiated incompatible pollen will be carried out for ascertaining the possibility of detecting S mutations.

1.4. Pollen tube growth was observed by means of fluorescence techniques after compatible and incompatible pollinations. These techniques allowed the observation of the bursting process of the self-incompatible pollen tubes in the style and confirmed the electron microscopy analysis (see the report by the "incompatibility group" of the Euratom-Ital Association). With this method, we were also able to detect after semi-compatible pollinations, the presence of one common S-allele in the two mating partners.

1.5. In vitro culture techniques gave us the possibility to save, by means of embryo culture, some interspecific hybrids between L.esculentum and L.peruvianum. These hybrids will be of great utility in our future researches.

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici:

A.Brunori, M.Devreux.

Titolo del progetto: Research at the macromolecular level of the modifications induced by in vitro culture in different plant tissues.

2.1. In vitro cultures of haploid tobacco pith were performed for finding out the modalities through which this very specialized tissue transit to a callus in proliferation. The observations were centered on DNA synthesis, on the presence of mitosis and on the cellularisation phenomenon. DNA was labelled by H^3 thymidine during two hours at $2 \mu C/ml$. Before fixation and after labelling, the pith explants were maintained for 24 hours in the culture medium in order to ensure incorporation by H^3 thymidine. (Preliminary experiments had shown that without this rest period between labelling and fixation, H^3 thymidine was not incorporated). The observations, made on histological sections, showed that labelled nuclei (2-3 %) were already present after 24 hrs and that their number increased to ± 25 % after two days of culture. The first mitoses appeared on the third day while after seven days, neoformations were well visible. The first cell layers around the pith explant underwent numerous subdivisions. These very large pith cells progressively transformed themselves into a large number of small meristematic-like cells. The central part of the explant degenerated while in the neoformed tissues, the first provascular cells appeared more and more abundant. This research was slackened by microdensitometric measurement difficulties and by the departure of Dr.Brunori who left the laboratory in june 72 (one year fellowship

at the laboratory of Dr.H.Stern at the La Jolla University in California for improving his knowledge in macromolecular biochemistry). The laboratory equipement was completed this year with a new Leitz MPV microcytophotometer.

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici:

M.Devreux, D. de Nettancourt, U.Laneri.

Titolo del progetto: Genetical studies on isogenic lines of tobacco derived from anther cultures and mutagenic treatments of the microspores from a diploid isogenic line.

3.1. Agronomic value of the tobacco isogenic lines derived from haploids obtained by anther in vitro cultures.

Nine isogenic lines of the cv. Perustitza, nine isogenic lines of the cv. Xanthy-Yaka and four isogenic lines of the cv. Erzegovina were compared to their respective controls at the "Istituto Scientifico Sperimentale per i Tabacchi" in Lecce. For each isogenic line, approximately five hundred plants were planted and fifty were analysed for calyx length, style length, length of the longest stamen and plant height. The lines were also scored during leaf-ripening for fresh and dry weight of the leaves, date of flowering and yield capacity. The general conclusions of these trials were that all the isogenic lines showed a very high level of uniformity but differed from one another for leaf shape and for the type of inflorescence. These lines proved to be perfectly adapted to the pedoclimatic conditions of the experimental Station, considered as a highly estimated material for their merchantable qualities, and were certainly superior to their respective controls. With regard to differences between isogenic lines, a Duncan test made on the data of plant height showed that two lines are smaller in Perustitza than the control while three are similar and four are higher; in Xanthy-Yaka, one line is smaller, four are similar and four are higher than the control; in Erzegovina, finally, two are smaller and two are higher than the control.

In the case of yield estimates (100 kilos of dry

weight per hectare), the control value for Perustitza amounted to 17.51 and was outflunk by three isogenic lines which released productions of 18.15, 18.98 and 19.10 respectively. Similarly, the cultivar Xanthy-Yaka (control value: 11.20) was outyielded by its best isogenic line (12.50) and the cv. Erzegovina (control value: 15.77) was inferior to two isogenic lines which yielded 16.41 and 16.98 respectively.

Also with the cv. Burley, six isogenic lines derived from anther cultures were analysed in the experimental fields of the A.T.I. Station near Caserta. Again, these isogenic lines displayed a good uniformity and were considered as excellent material for tobacco breeding. New isogenic lines of the cv. Burley were obtained in 1972 from three selections chosen among the most interesting for their agronomic values.

Finally, from the spontaneous photoperiodic mutant "Mammoth Kutsaga" of the cv. Virginia Bright, various isogenic lines were obtained and their agronomic analysis will be performed next year.

Biometrical analyses performed on F_1 derived from reciprocal crosses between two isogenic lines showed that the F_1 generations were uniform while the F_2 progenies expressed a very high variability. It is therefore obvious that the very high uniformity of isogenic lines is also present in their F_1 heterozygous progeny and such a characteristic is extremely interesting because it opens the possibility to produce large amounts of valuable F_1 hybrid seeds and to control very efficiently their production.

3.2. Mutated haploid plants obtained after microspore irradiation.

Flower buds from an isogenic line of the cv. Xanthy Yaka were irradiated with X-rays at two different ranges of total exposures (5, 10 and 20 R; 500, 1000 and 2000 R).

After anther culture, 119 haploids were analysed in the greenhouse. This number of plants analysed was so small that it was not possible to draw any conclusion on dose mutation relationships and this is, by far, the main limitation of the method. Yet, it is clear that the low doses (average of 11.7 % of induced mutations) were at least as effective as the high doses (7.1 %). This conclusion could be accounted for by the hypothesis that most of the mutations induced at high doses resulted from chromosomal aberrations and were lethal at the haploid level.

An attempt was also carried out to induce mutations in the anthers from an isogenic line of a real diploid species (*Nicotiana sylvestris*). Unfortunately, although haploids from this species were produced in very large amounts, all tentatives to obtain diploid isogenic lines by stem disk techniques have failed to date, all regenerated plants being triploid. It seems that the only possible explanation to the origin of these triploid plants must be based on the occurrence of somatic hybridisation between cells of the haploid tissue in culture.

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Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung mbH, München

Nummer des Vertrages: 118-72-1 BIO D

Leiter der Forschungsgruppe:

Prof. Dr. H. Gaul, Leiter der Abteilung Pflanzengenetik

Allgemeines Thema des Vertrages:

Cell-culture genetics in barley, wheat, potato and maize

This method should have important advantages in comparison with traditional breeding methods: Mutants can be detected in the M_1 -generation; cross-breeding work will be accelerated because F_1 hybrids from anther culture and subsequent polyploidization are already homozygotes and could result immediately in new varieties.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:

Prof. Dr. H. Gaul
Dr. G. Corduan

Titel des Projekts:

Development of techniques for the production of haploid plants through the use of anther culture

Darstellung der Ergebnisse:

The summary is a survey of used species and varieties, their pollen-development stage, number of cultured anthers, culture medium and other culture conditions.

No.	Species (no. of varieties)	Pollen stage	No. of anthers	No. of culture media	Culture con- ditions / Light / Temperature
<u>(A) Anther culture</u>					
(1)	Barley (16)	T,1,2,3	55.700	18	DL/25 ⁰ , DD/15, 18,25 ⁰ WL 16:8/28:21 ⁰
(2)	Maize (1)	1,2	7.500	12	DD/25 ⁰
(3)	Potato (18 = 4n) (48 = 2n)	T,1,2	34.080	36	DD/22,25,28 ⁰ DL/25 ⁰ WL 16:8/28:21 ⁰
(4)	<u>Digitalis</u> (1)	T,1	3.200	8	DD/25 ⁰ , DL/25 ⁰
(5)	<u>Convallaria</u> (1)	1	12.600	28	DD/25 ⁰ , DL/25 ⁰

Moreover, anthers of Verbascum were cultured.

(B) Callus culture

(3)	Potato			50	DD/25 ⁰ WL 16:8/28:21 ⁰ WL 14:10/30:20 ⁰
(4)	<u>Digitalis</u>			50	DL/25 ⁰ , DD/25 ⁰ WL 16:8/28:21 ⁰ WL 14:10:30:20 ⁰

The following culture media were used: Clapham, Fujii, Nitsch, Murashige & Skoog, Niizeki, Harn, Vasil, Nishi & Mitsuoka in anther culture, Winton, White, Wurm and Linsmaier & Skoog in callus culture.

With the described procedures we succeeded in Digitalis purpurea. After four weeks callus developed. Later on roots were formed.

Cytogenetical observation revealed haploid chromosome numbers. Finally we succeeded even in inducing organ differentiation.

In one tetraploid and eleven diploid strains of potato callus formation was confirmed. Furthermore root growth could be established in the tetraploid strain.

We could not induce callus up to now in barley, maize and Convallaria. So we started to test callus formation of seedlings and stems of barley varieties. Varieties, which show best callus formation, will be used for anther culture.

Jahresbericht über die Durchführung des Forschungsprogrammes "Laboratoriums-
und Massenzucht der Kirschenfliege sowie über deren Ökologie und Sterilisie-
rung"

A. Haisch

Vorbemerkung:

Die Kirschenfliege (*Phagoletis cerasi* L.) ist eine Trypetide des gemäßigten Klimas. Sie tritt vorallem in Europa und in einigen Teilen Asiens auf. Die Biotope des oligophagen Schädlings decken sich beinahe mit den Kirschenanbaugebieten. Eine Bekämpfung des Schädlings ist im Kirschenanbau mit großer Regelmäßigkeit durchzuführen, wenn das Erntegut nicht völlig befallen werden soll. Wegen des raschen Reifungsverlaufes der Kirschen und des auf die Ernte meist unmittelbar folgenden Konsums besteht bei der chemischen Bekämpfung eine erhebliche Gefahr, daß die Insektizidrückstände die gesetzlich vorgeschriebenen Toleranzen überschreiten. Aus diesem Grunde wird versucht, die Kirschenfliege genetisch zu bekämpfen.

Arbeitsprogramm:

Es besteht die Absicht, durch Aussetzen von Fliegen mit dominanten Letalfaktoren das Vermehrungspotential der Population so zu schwächen, daß sich Bekämpfungsmaßnahmen für die Dauer einiger Jahre erübrigen. Ausgehend von diesem Ziel ergeben sich folgende Aufgaben:

- 1.) Ernährungs- und entwicklungsphysiologische Untersuchungen haben die Grundlage für eine Massenzucht zu erbringen.
- 2.) Bestrahlungsversuche haben zu zeigen, inwieweit dominante Letalfaktoren ohne nennenswerte Beeinträchtigung der Vitalität der Fliegen erzeugt werden können.
- 3.) In ökologischen Untersuchungen sind die Faktoren der Populationsdynamik festzustellen, um zu wissen, wie rasch sich eine Population bestenfalls vermehren kann und die Bekämpfungsschwelle erreicht.

Projekt Nr. 1 Laboratoriums- und Massenzucht

Frühere Untersuchungen haben ergeben, daß die Nährsubstrate zur Aufzucht der Larven nach drei Gesichtspunkten zu beurteilen sind: Zersetzungstendenz durch Mikroorganismenbefall, Nährstoffgehalt und physikalische Beschaffenheit.

Nach den Versuchserfahrungen bilden wilde Hefen vor allen anderen Mikroorganismen eine Hauptgefahr für die nährstoffreichen Nährsubstrate der Larven. Eine Reihe von Antibiotika erwies sich zwar als höchst wirksam zur Unterdrückung dieser Hefen, leider aber waren sie auch larvizid. Ebenso unbrauchbar waren die verschiedenen p-Hydroxybenzoate (Nipa-Ester). Eine Anwendung von 0,4 % Propionsäure bei einem Säuregrad des Nährbreies von pH 4,0 - 4,2 löste indessen das Problem. Keines der zahlreichen anderen, getesteten Mittel oder deren Kombinationen erreichte die Wirksamkeit der Propionsäure.

Von den synthetischen und natürlichen Stoffen, die ernährungsphysiologisch für die Larven von Bedeutung sind, erwiesen sich Zucker, Hefe und Weizenkeime als wesentlich. Weitere Zusätze an Vitaminen, Mineralstoffen und Eiweißhydrolysaten brachten keine offensichtliche Verbesserung von Wachstum und Entwicklung. Unmittelbar tödlich wirkten sich Überkonzentrationen von Eiweißstoffen oder geringe Zugaben von Fetten oder Ölen aus. Durch abgestufte Konzentrationen wurde nun der optimale Gehalt von Zucker, Hefe und Weizenkeimen ermittelt, wobei als Maßstäbe für die Brauchbarkeit des Nährsubstrates die Puppenausbeute, die Puppenmortalität (3 - 5 Wochen nach der Verpuppung) und das Puppengewicht gewertet wurden. Die Menge an verabreichtem Zucker beeinflusste die Larvenentwicklung stark. Die natürlichen Gehalte der Hefe und der Weizenkeime an Zuckern erlaubten keine ausreichende Larvenentwicklung. Als optimale Konzentration hinsichtlich der Ausbeute und des Gewichtes der Puppen ergaben sich 4 %. Dagegen konnte kein deutlicher Einfluß auf die Puppenmortalität festgestellt werden.

Torulahefe wurde in folgenden Anteilen (%) verabreicht: 1,7, 3,3, 5,0, 6,7 und 8,3 %. Eine deutliche Beziehung zwischen Hefemenge und Puppenausbeute war nur insofern zu beobachten, als bei dem niedersten Gehalt die Puppenausbeute nur 20,6 % betrug, bei den übrigen Konzentrationen aber zwischen 27,3 und 36,1 % schwankte. Völlig unbeeinflusst blieb dabei die Puppenmortalität. Das Puppengewicht aber zeigte einen deutlichen Anstieg von durchschnittlich 3,0 bis 3,6 mg parallel zum Anwachsen der Hefemenge.

Der Weizenkeimgehalt wurde in den Konzentrationen 0,0, 1,0, 2,0, 3,0, 4,0 und 4,8 getestet. Sowohl die Puppenausbeute als auch die Puppenmortalität

wuchsen mit steigender Weizenkeimkonzentration. Als Optimum ergaben sich 4 % Weizenkeime, wobei 32 % der Larven sich verpuppten und von diesen Puppen wieder 36 % zu Grunde gingen.

In einer Reihe weiterer Versuche konnte festgestellt werden, daß schonend getrocknete und hitzegetrocknete, pulverisierte Kirschen keine ausreichende Ernährungsbasis für die Larven bilden. Eine Entwicklung war nur mit einer Spur von Hefe (1 %) möglich. Ein Hefeersatz durch Casein war ebenfalls unwirksam und ebenso ein Ersatz durch Casein mit einer Vitaminmischung.

Neben antimikrobiellen Substanzen und Nährstoffen wurde auch die physikalische Beschaffenheit des Nährsubstrates untersucht. Mehrere Faktoren sind hier von Bedeutung. Ihr Einfluß sollte durch veränderte Agar-Agarkonzentrationen (1, 2, 3, 4 und 5 %) festgestellt werden. Es ergab sich, daß die Puppenausbeute mit steigender Agarkonzentration zunahm, während die Puppenmortalität fiel. Ein optimaler Gehalt war 5 % Agar-Agar mit einer Puppenausbeute von 45 % bei einer Sterblichkeit von 34 %.

Projekt Nr. 2 Ökologie und Sterilisierung

a) Ökologie

Als methodische Vorarbeit für die ökologischen Untersuchungen wurde ein Verfahren zur Markierung von Fliegen ausgearbeitet. Hierbei nahmen die schlüpfenden Fliegen durch ihr Ptilinum markiertes Kieselgel auf. Als Tracer wurden Dysprosium und Samarium verwendet, die dann durch eine Neutronenaktivierung analysiert wurden. Das markierte Kieselgel war mit 10 % (Gew./Gew.) zu Quarzsand als Trägersubstanz gemischt worden. Das Verfahren erwies sich für die Versuche als brauchbar, wenngleich die Analyse sehr arbeitsaufwendig ist. Durch Aussetzen von Fliegen, die auf diese Weise markiert worden waren, wurde die Ausbreitungstendenz der Kirschenfliegenpopulation untersucht. An zwei verschiedenen Versuchsstellen zeigte sich, daß die ausgesetzten Fliegen sich innerhalb eines 90 m Bereiches im Verlauf von 1 - 2 Wochen verteilten.

Diese Versuche ließen auch grobe Schätzungen der Populationsdichten nachfolgender Berechnung zu:

$$P_0 = P_1 \frac{x}{y}, \text{ wobei die Buchstaben folgende Bedeutung haben:}$$

errechnete Population der unmarkierten Fliegen	=	P_0
Population der ausgesetzten Fliegen	=	P_1
Zahl unmarkierter, gefangener Fliegen	=	x
Zahl markierter, gefangener Fliegen	=	y .

Es ergab sich dabei eine Populationsdichte von 1900 Fliegen pro Baum in einem und 1000 Fliegen pro Baum im anderen Versuchsort.

In einer weiteren Analyse dieser Versuchsergebnisse zeigte sich ferner, daß die Fliegen sich nicht gleichmäßig innerhalb einer Kirschenanlage ausbreiten. Bäume von später Reifezeit und solche von starkem Fruchtbehang werden bevorzugt angefliegen.

b) Sterilisierung

In Bestrahlungsversuchen mit Hilfe einer Cäsiumquelle (66 R/Min.) wurde die Sterilisationsdosis für männliche Kirschenfliegen ermittelt. Nach Applikation von 8000 R im späten Puppenstadium sank die Lebensfähigkeit der Fl-Generation unter 1 %. Weibchen, welche diese Dosis erhalten hatten, legten in der Regel keine Eier mehr.

Bei diesen Versuchen ist die Erhaltung der Vitalität der Fliegen ein Hauptproblem. Bei den bestrahlten Puppen handelte es sich um solche, die aus befallenen Früchten gesammelt worden waren. Das bedeutet sehr heterogene Entwicklungsstadien zum Zeitpunkt der Bestrahlung. Manche Fliegen schlüpfen erst drei Wochen nach der Bestrahlung. Es ist klar, daß diese Fliegen starke somatische Schäden durch die Bestrahlung erlitten haben. Es muß deshalb versucht werden, diesem Mangel durch eine Bestrahlung im Imaginalstadium abzuhelpen.

Zusammenfassung

1. Die Versuche zur künstlichen Ernährung der Kirschenfliegenlarven brachten eine erhebliche Verbesserung des Aufzuchterfolges. Dieser Erfolg beruht auf der Lösung des Mikroorganismenproblems, auf der Verbesserung der Nährstoffversorgung und auf der günstigeren physikalischen Beschaffenheit des Nährsubstrates.
2. An populationsdynamischen Faktoren wurden die Ausbreitungstendenzen zweier Populationen und Ursachen ihrer Beeinflussung untersucht. Ebenso wurden Populationsdichteschätzungen durchgeführt.
3. Bestrahlungsversuche ergaben eine Dosis von 8000 R, welche zur Sterilisation von Kirschenfliegenmännchen genügt. Ferner wurde das besondere Problem der Vitalitätseinbuße durch die Bestrahlung kurz erläutert.

RADIOENTOMOLOGIE

RADIOENTOMOLOGY

RADIOENTOMOLOGIE

Associato alla Commissione: Istituto di Entomologia agraria della Università di Padova.

N° del contratto: 105-72-1 BIOD I

Capo del gruppo di ricerca: Prof. Sergio Zangheri

Tema generale del contratto: Ricerche sulle metodologie e le tecniche per l'allevamento in laboratorio del Dacus oleae Gmel. e di altri insetti di interesse agrario in vista della produzione in massa e ricerche radioentomologiche e biologiche, con particolare riguardo ai ditteri tripetidi.

General Theme of the contract: Researches on methods and techniques for laboratory rearing of Dacus oleae Gmel. and other insects of agricultural interest for mass-production and radioentomologic and biologic researches with particular regard to fruit flies.

A brief outline of the work carried out in 1972

While executing the project 1, a comparative examination of bacterial symbiosis has been set up in different fruit flies extending the one of Dacus oleae Gmel. already in progress. The anatomical study has shown the praryngeal vesicle steadily present in all investigated species and, from what is possible to state, symbiont bacteria present in the vesicle of Ceratitis capitata Wied.

Researches concerning project 2 have pointed out some growth factors necessary for breeding various leaf roller on synthetic media. It has been possible to ascertain the positive action of steroids and fatty acids; moreover not yet definite data have been so far gained, which consequently are being checked, on the dietetic significance of other substances from vegetal source.

The bio-ecological study of *Dacus oleae* Gmel. project 3 has been developed in three main directions.

An investigation on the activity of *Dacus oleae* adults of populations living around the lake of Garda, a preliminary study for the researches on the biology and the dynamics of populations, has made it possible to fix the thermic range within which the adult can disperse, feed and reproduce. Hence it follows that during winter, too, in the Garda lake area the adult insect finds climatic conditions generally suitable for a frequently active survival, lowest temperatures not reaching lethal values.

The biological cycle includes four generations, while wintering prevails at the adult stage.

The capture of adults have been remarkably improved by means of chromotropic traps. This fact has allowed to make one first release and recapture test of marked individuals and to gain excellent sampling results.

Within the limits of project 4, besides carefully examining the radiosensibility of *Dacus oleae*, researches have been made on the effect of different doses of gamma radiations on *Opius concolor* Szepi., an important parasite of same *Dacus*. Comparative tests between gamma radiations and chemosterilizers have concerned *Ceratitis capitata*. Investigations have been begun on the radiosensibility of insects harmful for food-stuffs.

The researches of project 5, made on the ground of a large number of sterilization experiences with different doses, have pointed out the best ratio of sterile males to one fertile pair in *Dacus oleae* (the relative mathematical model is in elaboration). This has been confirmed by a first restricted field test. Moreover the cause of the prevailing monogamy in *Ceratitis capitata* has been studied by transplanting parts of the reproduction organs, awaiting to extend then same researches to *Dacus oleae*.

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami.

Titolo del progetto: Influenza dei batteri simbiotici sul ciclo vitale del Dacus oleae Gmel.

The influence of symbiotic bacteria on life cycle of Dacus oleae Gmel.
(Researches made in 1972)

The research was first directed to analyse bacterial symbiosis in the chief fruit flies: Dacus oleae Gmel. and, as a comparison, Ceratitis capitata Wied., Dacus cucurbitae Coquillet, Dacus tryoni Frogg. Dacus dorsalis Hendel, and others. The research, was carried out on anatomical and histological bases and was intended to show whether the type of symbiosis of D.oleae was unusual or included in a pattern common to the above mentioned fruit flies. This all was done in order to obtain possible information on the future surviving of symbionts in the case of insects grown on an artificial medium and consequently on the possible importance of the fact for the successful rearing.

Up to this time besides in D.oleae, as already known, the first results have also shown the presence of the pharyngeal organ, the seat of symbionta, in D.tryoni and in C.capitata. This fact has also made it possible to recognize, as a similar structure, a feature described in a contribution by R. W. DEAN (1933), unexplained by the Author.

Besides this, a microbiological investigation of the symbionts of D.oleae and of C.capitata has been set up: at present it is possible to state that in the latter fruit fly also bacteria come out from pharyngeal vesicle and cross the tract of the following alimentary canal appearing by the midgut as agglutinations of individuals covered with a mucilaginous capsule.

When examined through the microscope the bacteria living with the two species prove morphologically different.

Researches are carried on in the direction already stated in the following work, a result of the previous collaboration of the Institute of agricultural Entomology in Padua University with the Section of Entomology of C. C. R. Euratom of Ispra.

GIROLAMI V., and CAVALLORO R. - Some aspects of bacterial symbiosis of Dacus oleae Gmelin in field and in laboratory rearing. Ann. Soc. Ent. Fr. (N.S.) 8 (2), 1972.

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami, Dott. ssa L. Panizza.

Titolo del progetto: Ricerche su substrati artificiali per gli stadi larvali del Dacus oleae Gmel. e di lepidotteri defoliatori e sulle tecniche di allevamento.

Researches on artificial media for the larval stages of Dacus oleae Gmel. and of phytophagous Lepidoptera and on rearing techniques. (Researches made in 1972)

Current rearing of the leaf roller Choristoneura lafauyana Rag. has been aided by the rearing of similar insects, Archips podanus Scop. and Adoxophyes reticulana Hb. A large number of comparative tests have been made in order to ascertain growth factors from vegetal origin necessary to the larval development of the species examined.

In fact the artificial media employed contain chemically undefined vegetal products, as the wheat germ, or as the case is with our rearing, one commercial chlorophyll extract.

Determining the exact chemical nature of nutrient principles is absolutely required both for increasing rearing yield and chiefly for planning mass-production of insects. In this way efforts are made to remove yield inconsistencies due to variations of active principles contained in commercial products, nature and quantity of which are unknown.

Experimentations carried out employing chemically definite substances have made it possible to ascertain a basic action of steroids and fatty acids on growth; investigations are also carried on to check the influence of other vegetal compounds, among which tannines and carotenoid and pyrolic pigments.

The first results achieved in this field have already been published (GIROLAMI V., 1972 "The influence of vegetal pigments on the larval growth of phyllophagous lepidoptera", Atti e Mem. dell'Accad. di Sc. Lett. ed Arti, vol. LXXXIV, parte II, classe "Scienze matematiche e naturali", pp. 149-158).

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici: Prof. S. Zangheri, Prof. L. Masutti, Dott. V. Girolami.

Titolo del progetto: Ricerche sulle cause di mortalità del Dacus oleae Gmelin e sulla dinamica delle popolazioni in natura ed in laboratorio.

Researches on mortality causes of Dacus oleae Gmelin and on population dynamics in field and in laboratory.
(Researches made in 1972)

A. The survival of Dacus oleae adults in laboratory proved to be dependent, to a great extent, on light intensity and distribution in the surrounding space. In fact it is evident that if light radiation comes from one direction only insects frantically seek together in the best lighted places and while doing so they very soon exhaust their strenghts, even failing to feed. As a consequence survival is drastically reduced.

Air moisture influence has not proved as important, because its values do not exhibit a significant difference from the wide tide range observed in field. Only when solid and hygroscopic foods are used to feed Dacus adults, it is necessary to raise relative humidity remarkably, in order to help food uptake.

As to temperature, researches have found out that D. oleae adults do not bear values exceeding +40°C. The lowest values recorded in the Gargano olive area (western coast of the lake of Garda), equal to -3°C, are endured for a long series of nights.

A further fall in temperature is the cause of an increasing rate in mortality; death takes place within some ten minutes at -10°C. The trophic activity of adult Dacus however is started only at a body temperature of +7°C and the flight, necessary for searching food sources, begins at +15°C: so these are the thermic values of decisive importance for winter survival of the adult insect, that must feed at least at intervals during the cold season.

B. In the area of the lake of Garda D. oleae reaches four generations in one year. The first, at the appearance of the new olives, from July to September; the second, from September to the following March; the third, in spring, from March to May on surviving olives; the fourth, again on surviving olives of late varieties, in May-June. The adults of the last generation are responsible for the attacks to the new olives at the end of July.

During all winter time Dacus adults are found in field, which leads to think that this stage is entrusted with the species survival in the areas investigated. After the beginning of the cold season no adults have so far been obtained from the pupae wintering in the soil. At present investigations are in course on the possible development of spring flies from the few larvae found in olives till the end of January.

The steady improvement of capture systems by means of chromotropic traps has greatly helped sampling adults. In winter time however it is impossible to obtain a significant number of Dacus, owing to the torpor of the adults, which do not show a sufficient flight activity until their bodies reach a temperature of +17°C. In the area of the lake of Garda this situation occurs quite seldom even in the sun during cold months.

Moreover very important is the flight stop caused by the feeblest wind, which cannot be recorded by current meteorological instruments. For this reason the behaviour of D. oleae is now being studied in an area well-protected against wind.

A comprehensive investigation has already been started on the amount of the fly autumn populations in all the Gargnano territory. This is feasible because of the geographic isolation of the same territory, due to natural barriers breaking off the continuity of olive-grown areas.

C. The efficacy in the system of sampling adults by means of chromotropic traps has made it possible to carry out an experience of autumn recapture of individuals marked with fluorescent pigments. The test proved highly successful: recovery of adults has reached 34% and has supplied with necessary information both on the amount and trend of fly displacements, and on the density of natural populations.

Tab. I SOPRAVVIVENZA ALLE ALTE TEMPERATURE DEGLI ADULTI
DI DACUS OLEAE GMELIN

(Survival of Dacus oleae Gmelin at high temperatures)

Temperatura (C°±0,5)	MORTALITA' IN GRUPPI DI 10 ♂ E 10 ♀											
	Alimentazione	1					2		3		4	
		1/2	1	2	4	8	matt	sera	matt	sera	matt	sera
43	completa	15	19	20								
	sola H ₂ O	5	19	19	20							
	nulla	20										
42	completa	9	13	19	20							
	sola H ₂ O	3	5	18	20							
	nulla	19	20									
41	completa	4	6	8	9	10	17	20				
	sola H ₂ O	2	5	5	8	9	15	18	20			
	nulla	15	19	20								
40	completa	2	5	5	8	15	16	18	20			
	sola H ₂ O	2	5	8	8	8	13	17	18	20		
	nulla	18	15	20								
39	completa	0	0	3	5	12	15	17	18	20		
	sola H ₂ O	2	5	6	10	10	14	16	19	20		
	nulla	0	2	18	20							
38	completa	0	0	0	0	2	2	2	2	2	4	8
	sola H ₂ O	0	0	0	0	0	6	19	20			
	nulla	0	0	4	16	20						
37	completa	0	0	0	0	0	0	0	0	0	0	0
	sola H ₂ O	0	0	0	0	1	7	16	16	18	20	
	nulla	0	0	3	11	20						
36	completa	0	0	0	0	0	0	0	0	0	0	0
	sola H ₂ O	0	0	0	0	0	6	13	15	19	20	
	nulla				3	16	20					
35	completa	0	0	0	0	0	0	0	0	0	0	0
	sola H ₂ O					0	1	3	11	16	19	20
	nulla					4	17	19	20			

Mortalità degli adulti di D.oleae a varie temperature (senza cibo, o con sola acqua, o con cibo liquido ed acqua). Prove in termostato a 1000 lx ed U.R. superiore al 55%.

Mortality of D.oleae adults at different temperatures (kept without food, or supplied with water only, or with liquid food and water). Tests in thermostate at 1000 lx and relative humidity over 55%.

Tab. II SOPRAVVIVENZA ALLE BASSE TEMPERATURE DEGLI ADULTI
DI DACUS OLEAE GMELIN

(Survival of Dacus oleae Gmelin at low temperatures)

Temperatura (C° ± 0,5)	MORTALITA' IN GRUPPI DI 10 ♂ E 10 ♀ ore								
	1/2	1	2	3	4	5	7	10	12
-20	20								
-15	20								
-10	16	20							
- 9	1	5	20						
- 8	0	0	1	3	19	20			
- 7	0	0	0	1	12	17	20		
- 6	0	0	0	0	3	8	18	20	
- 5	0	0	0	0	4	8	16	19	19
- 4	0	0	0	0	0	0	0	10	11
- 3	0	0	0	0	0	0	0	0	1
0	0	0	0	0	0	0	0	0	0

Mortalità degli adulti di D.oleae dopo una esposizione a varie temperature al buio (per simulare la notte) e con U. R. superiore al 70%.

Mortality of D.oleae adults after a steady exposure to different temperatures in the darkness (to simulate the night) and with relative humidity over 70%.

Risultati del progetto n. 4

Capo del progetto e collaboratori scientifici: Prof. R. Cavalloro, Dott. G. Delrio.

Titolo del progetto: Effetti biologici comparati delle radiazioni gamma, neutroni e chemiosterilizzanti sui diversi stadi di vita degli insetti.

Comparative biological effects of gamma radiations, neutrons and chemosterilants on different life stages of insects.

(Researches made in 1972)

A. A careful study was made on radiosensitivity to gamma radiation in all growth stages of Dacus oleae Gmel., which is of basic importance for a better use of ionising radiations for sterilization the above said insect.

B. An investigation was carried on the influence of radiations on the adult of the Braconid Hymenopterous Opius concolor Szep., an important parasite of Dacus oleae Gmel., also in order to find possible different effects on host-parasite.

The adults of Opius concolor Szep. were radiated at the end of their first day of life and placed in groups of 200 in plexiglass boxes with adequate food. Three replications were made for each thesis; mortality was recorded daily; fertility was checked by exposing mature larvae of Dacus to irradiated females of Opius. With the exception of the dose of 10 krad, all other doses used greatest increased mortality in comparison with the control ones (Table 1). The act of egg-laying continues until 50 krad, although fertility is missing with all doses.

Further researches are carried on in order to determine sterilizing doses and radiosensitivity in other life stages of the insect.

C. Tests have been started on comparative biological effects between gamma radiations and three chemical compounds (aminopterine, bromodioxyuridine, iododeoxyuridine), in Ceratitis capitata Wied.

D. At last, some researches were begun on the radiosensitivity of the species of the most harmful insects for preserved foodstuffs.

Tabella 1. - Effetti di dosi diverse di radiazioni gamma sulla longevità degli adulti di Opius concolor Szep.
(valori medi relativi a 100 coppie, con 3 replicazioni).

DOSE (Krad)	MORTALITA' ACCUMULATA (%) DOPO GIORNI													
	2	4	6	8	10	12	14	16	18	20	22	24	26	28
0	0,6	3,8	5,0	10,4	16,3	26,8	43,1	56,6	71,1	80,6	94,1	98,4	99,5	100,0
10	0	2,7	6,3	6,3	14,4	20,7	31,5	37,8	67,5	77,4	86,4	97,2	99,0	100,0
20	0	5,8	13,5	16,5	16,5	23,3	40,7	62,1	100,0					
30	1,1	8,2	11,7	16,4	28,2	64,7	87,0	100,0						
50	1,0	2,0	4,1	16,6	54,1	97,9	100,0							
100	0	12,5	37,5	100,0										
150	0	13,3	100,0											
300	100,0													

Risultati del progetto n. 5

Capo del progetto e collaboratori scientifici; Prof. R. Cavalloro, Dott. G. Delrio.

Titolo del progetto: Studi sul comportamento di femmine e maschi normali e sterili.

Studies on the behaviour of normal and sterile females and males.
(Researches made in 1972)

A. Starting from the effects of ionizing radiations on Dacus oleae Gmel. directly recorded in previous investigations, tests were made on the behaviour of the radiosterilized insect.

As the doses for sterilizing adults (15 krad) are twice as high as those necessary to induce over 98% of dominant letals, radiating the pupal stage (8 krad), which leads to get adults living for a shorter time, it is advisable to sterilize insects at the pupal stage. These latter do not live shorter than untreated insects, males however transmit their spermatozoa only while mating the first times, then they keep pairing although they are aspermic. Although during this period they do not actually transmit sperms, they cause a non-receptivity for other matings in a percentage of females.

Females mating more than once reduce their fertility if one pairing occurs with a sterile male; besides, in order to establish the fertility degree, it is of greater importance the kind of male (either sterile or not), wich mated last. In any case it seems that the sterile sperm is less competitive if compared with the fertile sperm.

The best ratio of sterile males to fertile pair to be used in the genetic control proved to be 8:1:1 in tests carried out in laboratory. This figure was subsequently confirmed in field experiments by freeing sterile males to defend caged olive trees.

The results of the above mentioned tests were reported at the 14th International Congress of Entomology, Canberra (22nd-30th August 1972) and a publication is in the press on the "Journal of Economic Entomology" (enclosure n.1).

B. A considerable significance was attached to the research on causes of the prevalent monogamy noticed in Dacus oleae Gmel. females. To this purpose and in order to set up investigation techniques which are still unknown, researches were carried out, making use of the males of Ceratitis capitata Wied., a species with a similar behaviour and for which there was an availability of considerable laboratory material.

These insects were castrated with the mechanical removal of the testicles, and were mated with normal females: a high percentage of these latter, very close to the percentage of females mating with normal males, did not mate again, showing at least for the present the non-absolute necessity of transmitting spermatozoa in order to bring about monogamy.

Other tests are carried by grafting parts of male sex organs in virgin females.

C. A mathematical model for the practical use of the sterile male is under investigation for the control of harmful insects, with particular regard to competition between normal and sterile individuals.

Contract No. 115 - 72 - 1 BIO D

Institut für Genetik, Johannes Gutenberg-Universität, Mainz, Germany
Prof. Dr. H. Laven

Development of genetical control systems in pest insects.

Project No.1: Mosquitoes.

Previous investigations on the production and isolation of reciprocal translocations in several mosquito species (Culex pipiens, C. tritaeniorhynchus, Aedes albopictus, A. caspius) have shown that such chromosomal aberrations can be recovered easily and in high yield after X-ray irradiation. The programme carried out by the Institute in 1972 was aimed at the further development of translocations and complexes with several different translocations in order to get systems with higher degrees of sterility. For that purpose systems with two or three translocations in one line have been induced. Other translocations were tested for the possibility of homozygotisation. Several translocations were combined by crosses and in this way balanced lethal systems have been constructed which lead to a reduction of female offspring. The same was also achieved by the combination of recessive lethal factors and a translocation. Part of these translocation complexes and a series of female-linked translocations have been investigated cytologically and their nature as these types of chromosomal aberrations has been confirmed.

As a preliminary step for the use of translocations for control in the field, several laboratory cage and field cage experiments were made with an integrated strain of Culex pipiens, i.e. a strain with incompatibility and a high degree of sterility due to a translocation complex. The results are promising in spite of the fact that a slight tendency for assortative mating of the normal as well as translocation animals became obvious.

As a model for control of a pest species which cannot be bred in the laboratory half of the males of a stable cage population of Culex pipiens was irradiated in each generation. Due to dominant lethality the population decreased steadily in five generations to about 2% of the original size. At the same time the number of animals carrying a translocation increased to more than 80%.

Project No.2: Mediterranean fruitfly.

A culture of the mediterranean fruitfly has been established in the Institute. In preliminary experiments the intrinsic lethality of the strain was determined. After that irradiation experiments were initiated and a series of semisterile lines were isolated. These are presently under study.

Project No.1

Title: Genetical systems for control of mosquitoes.

Research workers: Prof.Dr.H.Laven, G.Merten, H.W.Müller, K.Orthlauf-Bloos, R.Pisarzewski, L.Reidel, P.Schindler, J.Schildknecht.

1.1. Development of strains of Culex pipiens with double translocations.

Males of a strain with a single male-linked translocation and an average sterility of 69% were subjected to irradiation for a second time with 2700 r X-rays. In the F₁ of these irradiated males 11 out of 96 individuals had higher sterility degrees between 79 and 89%, outside of the variability in the original line. By further inbreeding of the 11 lines with higher sterility 7 remained stable with average sterilities between 76 and 84%. In one line (DT 71) approximately 4.000 gametes have been tested for stability of the sterility degree and the underlying translocation complex. No exceptions were found.

A second experiment for the production of translocation complexes was conducted with a lower irradiation dosage (500 r X-rays) and with males of a strain with a lower average sterility (52%). In this case only three stable lines with higher sterility (70-80%) could be isolated.

1.2. Integration of translocation systems with incompatibility.

In the Culex pipiens species complex normal crossing barriers exist between allopatric populations due to an extrachromosomally inherited incompatibility which does not prevent copulation between such populations. This prefabricated "sterility" has already been used successfully in a field experiment in Burma. This control method has a serious disadvantage because only males can be released, otherwise a natural population would be replaced by a fully reproductive population with the crossing type of the released animals. Integration of incompatibility and a translocation complex with high sterility would lead to a strain with total sterility against a natural population and with such low reproductive potential that survival could be impossible. Such release measures would not need separation of the sexes and the integrated strain could probably be reared under natural conditions in separate breeding containers or together with the natural population in their breeding places.

Several such integrated strains have been developed and one of them has been tested in laboratory and field cage experiments for competitiveness (see section 1.6.).

1.3. Screening for viable homozygous translocations.

A series of 7 female-linked and 4 autosomal translocations, originating from an irradiation experiment with 2800 r X-rays, were tested for the possibility of making them homozygous. The attempt failed for the 4 autosomal translocations but 3 out of the 7 female-linked translocations could be made homozygous. One of these homozygous lines has almost normal viability and fecundity, the other two are somewhat weaker and less productive. But this lowering of viability does not prevent mass production. There is now the possibility to use these homozygous strains in pairs for the production of double heterozygous animals with a higher degree of sterility. In preliminary observations it has been found that the double heterozygotes show a heterotic superiority over normal animals and such with single translocations.

These investigations will be continued. A new experimental series has been started with a low irradiation dosage (500 r X-rays) in order to produce less recessive lethal factors in the translocated chromosomes and enhancing in this way the possibility to develop strains with homozygous translocations. 10 lines with autosomal or female-linked translocations are presently under investigation.

1.4. Balanced lethal systems with translocations.

As mentioned in the foregoing section out of 7 female-linked translocations only 3 could be made homozygous, the other 4 were lethal in homozygous condition. This lethality can be due to the lethal factors or deficiencies connected with the break points of the translocations or due to additional recessive lethal factors somewhere else in the chromosomes involved in the translocations. Since in mosquitoes the male is the heterogametic (M/m) and the female the homogametic sex (m/m), female-linked translocations which are not viable in homozygous condition can be used for the construction of balanced lethal systems affecting only the females. Such systems have been constructed with 3 of the 4 female-linked translocations.

By crossing normal males with females having one of the female-linked translocations (+/T^{m1}), males were selected which carried this translocation (+/T^{m1}). Crosses of such males with females carrying a second female-linked translocation (+/T^{m2}) gave now males with the second translocation (+/T^{m2}) and females with both translocations (T^{m1}/T^{m2}) besides females with one translocation (+/T^{m1}). The double translocated females (T^{m1}/T^{m2}) were selected and inbred with their brothers (+/T^{m2}). Half of the female offspring of this cross is not viable because of

homozygosity for one translocation (Tm^2/Tm^2), the other half carries both translocations (Tm^1/Tm^2). All subsequent generations will produce males with one or the other translocation ($+/Tm^1$ or $+/Tm^2$) and only about one third the number of females (Tm^1/Tm^2), because two thirds a nonviable (Tm^1/Tm^1 and Tm^2/Tm^2). With a lethality of $Tm^1=47\%$ and $Tm^2=49\%$, the double heterozygous females are 73% sterile. But as a consequence of the reduction of the number of females born, the total reproductive potential of such a strain, compared with a strain with the normal sex ratio of 1:1, is only about 10% .

By the inclusion of a male-linked translocation into the same system the number of females was reduced to $1/10$. The ensuing total sterility in this strain amounted to almost 98% .

Similar systems have been constructed with recessive lethal factors in normal chromosomes which lead to death of otherwise normal females carrying no translocation.

From these preliminary investigations it seems to become obvious that by combination of different deleterious genic and chromosomal aberrations strains can be constructed which have any desired degree of sterility up to almost total sterility.

1.5. Cytological investigations on translocations.

The main type of chromosomal aberrations in mosquitoes produced by X-irradiation and screened with the manifestation of partial sterility are reciprocal translocations. This has been verified by a limited amount of cytological investigation of pachytene configurations in spermatogenesis. The production and isolation of lines with enhanced sterility after a second irradiation (see section 1.1.) led to a cytological investigation also of the chromosomal aberrations. All lines with a high degree of sterility ($76-84\%$) have multiple translocations, some of them with rotational exchanges between all three chromosome pairs. But high degree of sterility as such cannot be regarded as evidence for multiple exchanges. Two lines have been found to possess only a single reciprocal translocation giving about 69% sterility. Other lines with a somewhat higher sterility (76%) are double translocation lines. The correlation between number and type of translocation, length of exchange and interstitial segments and the degree of sterility is presently not yet clear and needs further investigations.

The double translocation systems, especially the ones with rotational exchanges are very useful tools for chromosome substitution and for the development of homozygous translocations. Such investigations are planned for the near future.

1.6. Cage experiments for the evaluation of integrated strains.

As mentioned in section 1.2. strains have been constructed for Culex pipiens which have a certain crossing type (incompatibility) and contain a double translocation. One such integrated strain is incompatible in both crossing directions with a wild population in India (Delhi) and has 85% sterility (designation D3/71).

In a first cage experiment in the laboratory during 10 successive days 100 ♂♂ and 100 ♀♀ each of a normal Delhi strain and of the integrated strain D3/71 were released into a cage. Thus the release was in a ratio of 1:1. Theoretically 50% of the egg rafts produced by the females of both types should be sterile due to the bidirectional incompatibility, 25% should show full hatching and 25% of the rafts only 15% hatching. The actual figures were 40.36%, 30.34% and 29.30%. This indicates a certain and about equal degree of assortative mating of both types of mosquitoes. The second generation was set up according to the number of animals of both types born plus the same number of D3/71 animals as released in the first generation. The ratio of Delhi to D3/71 was now 1:2.47. Again assortative mating took place but due to incompatibility (28%) the population decreased. The third generation was built up in a ratio 1:12.94 and it increased to a ratio of 1:29.62 in the fourth generation. Accordingly in this generation the normal animals were almost exterminated and the D3/71 remained in a size of only of about 10% of the original population and would also have been exterminated in the sixth generation.

The foregoing experiment was conducted with the assumption that density dependent factors would not compensate for the reduction in population size. In a second experiment in an outdoor cage for every generation a five-fold increase was assumed. Furthermore, only one release of D3/71 in a ratio of 1:9 was made at the start and all following generations were built up according to the results times a five-fold increase. The population went steadily down from an initial size of 10,000 animals to 7,000, 4,690, 3,140, 2,100 etc., in spite of the assumed five-fold increase. Total extermination could be expected around generation 10-12. The initial ratio of Delhi to D3/71 was maintained during all following generations, therefore the continuous decline of the population. It must be emphasized that in this experiment releases of the D3/71 strain were made only once, the subsequent decrease of the population was therefore due to the inherited incompatibility and sterility.

1.7. Cage experiment with partial sterilization.

The sterile male technique has so far failed in mosquitoes, because total sterilization affects the males in such a way, that they become less competitive. It has been suggested by several authors that partial sterilization with a lower dosage would also lead to population reduction and possible eradication. Such lower irradiation would also produce a certain amount of inherited sterility, as has already been observed in certain Lepidoptera. We were interested to explore the effect of partial sterilization under two aspects, first, what amount of inherited sterility would be produced, and second, whether such a procedure could be used in mosquitoes or other pest insects which cannot be reared in the laboratory.

For this purpose in every generation half of the males of an initial population of 480 normal males and 480 normal females were irradiated with 5000 r X-ray producing about 89% dominant sterility and an average reduction of competitiveness of 18%. Under the assumption of population equilibrium (absence of density dependent factors) the population was reduced in the first generation to 66.27% of the original size, in the second generation to 38.89%, in the third to 14.99%, in the fourth to 6.28% and in the fifth to 2%. Dominant lethality is responsible for most of this steady reduction of the population. But concurrently with this reduction the percentage of animals with inherited partial sterility went rapidly up from an initial fraction of 28.94% in the second generation to 41.89% in the third, 60% in the fourth, 81.81% in the fifth to fully 100% in the sixth generation. This experiment shows clearly that partial sterilization can produce inherited sterility in ever increasing amount so that after several generations the release of sterilized males can be terminated and the population will automatically decrease by these systems. The advantage of such a method for control over total sterilization are obvious, because the latter method needs continuous releases until no more insects are born. It remains to be seen whether in other mosquitoes and pest insects the inherited sterility can be produced to the same extent as in the test species Culex pipiens. The application of this method to natural populations of insects which cannot be reared in the laboratory would depend not only on the degree of partial sterility produced but also on the size of that part of the population that can be obtained for irradiation treatment.

Project No.2

Title: Production of semisterility in the mediterranean fruitfly
(Ceratitis capitata).

Research workers: Prof.Dr.H.Laven, Dr.Y.Rössler

After some initial difficulties a colony of Ceratitis capitata could be established. The egg hatch in this colony was $92.9 \pm 6.4\%$, but the survival of larvae up to pupation ($26.3 \pm 9.5\%$) and adult emergence was rather low ($18.9 \pm 3.44\%$). The reasons for this poor survival are not known and need further investigations. Also the percentage of ovipositing females was rather low, probably due to unsuitable adult food. In spite of the various technical difficulties which might be overcome in the future, it could be demonstrated that semisterile lines can also be produced and isolated. Out of 71 ovipositing pairs in F₁ from irradiated males 32 pairs showed reduced egg hatching (21-71%). For only two lines out of the 32 data are so far available for the following generations. Line No.1 had a stable egg hatch of about 50% for four generations and line No.2 of about 56% for three generations.

The work with Ceratitis capitata has been interrupted because Dr.Rössler left the Institute for his home country, but it will be taken up again as soon as possibilities arise.

Contractant van de Commissie: Instituut voor Plantenziektenkundig
Onderzoek (I.P.O.), WAGENINGEN, Nederland

Nummer van het contract: 098 - 72 - 1 BIO N

Hoofd van het researchteam : dr. ir. J. Ticheler

Algemeen onderwerp van het contract : Bestrijding van de uievlieg,
Hylemya antiqua, (Meig.), met behulp van de "sterile-male" techniek.

Een tweede veldproef heeft aangetoond dat het loslaten van gesteriliseerde vliegen de voortplanting van de wilde populatie in evenredigheid met de verhouding steriele/fertiele vliegen verminderde. Dank zij een ruimtelijk meer gespreide loslaattechniek kon dit effect gedurende het gehele seizoen gehandhaafd worden. Het bleek moeilijk de vluchtcurve van de wilde populatie goed te volgen met het loslaten van steriele vliegen. Loslaten op een constant niveau blijkt wenselijk en zal in 1973 beproefd worden. De massaweek heeft voldoende vliegen van duidelijk verbeterde kwaliteit kunnen produceren. In het uieteelgebied werd de studie van de verspreiding van de uievlieg voortgezet. Van de verplaatsing van de vlieg is een simulatiemodel gemaakt waarin verschillende oriëntatieprikkele zoals aantrekking door uievelden, oriëntatie door windrichting etc. zijn ingebouwd. De uitkomsten van een proef waarbij gemerkte vliegen zijn losgelaten en teruggevangen zullen met behulp van dit model geanalyseerd worden. Ook zijn vele directe waarnemingen van het gedrag in het veld gedaan, waarbij o.a. dagritmen bestudeerd zijn.

Isotopen bleken een nuttig hulpmiddel voor verschillende aspecten van de studie. Met behulp van ⁶⁵Zn dat ook in de nakomelingschap van gemerkte vliegen aangetoond kan worden, kon de vermenigvuldiging in het veld worden nagegaan. Met behulp van ³²P werd aangetoond dat honingdauw door uievliegen als voedsel wordt gebruikt.

Het histologisch onderzoek werd voortgezet, waarbij bleek dat bestraling een volledige remming van de DNA synthese veroorzaakt in de germinale cellen van de gonaden, doch niet in de somatische cellen. Een begin is gemaakt met het vergelijken van de stralingspathologie van de uievlieg met die van een aantal andere insectesoorten. Een aantal aspecten van de stralingspathologie wordt nader electronenmicroscopisch onderzocht.

Studentenonderzoek leverde een belangrijke bijdrage op het gebied van het gedragsonderzoek en van de inductie en verbreking van de diapauze.

Publicaties:

- J. Theunissen : Egg chamber development in the onion-fly, *Hylemya antiqua* (Meigen) (Diptera, Anthomyiidae). Int. J. Insect Morphol. Embryol. ter perse
- J. Ticheler, M. Loosjes, J.Ph.W. Noordink, J. Noorlander and J. Theunissen: Field experiments with the release of sterilized onion flies, *Hylemya antiqua* (Meig.). Proc. FAO/IAEA Expert Panel on The Practical Use of the Sterile Male Technique for Insect Control (13-17 November 1972, Vienna). ter perse.

Project nr. 1

Bestrijding van de uievlieg, *Hylemya antiqua* (Meig.), met behulp van de "sterile-male" techniek.

Algemeen (J. Ticheler)

In 1972 was het werk van het uievlieg-team voor een belangrijk deel gericht op de uitvoering van de tweede loslaatproef met steriele uievliegen. Daarbij werd voortgebouwd op de gegevens die de eerste veldproef in 1971 had opgeleverd. Naast de vaste leden van het team werkten mee aan het verzamelen van de gegevens Mej. H. de Zwaan en de Heren Uyalo, R. v.d. Leeuw en G. Schelling.

De veldproef (J. Ticheler)

In hoofdzaak zijn de methoden gebruikt bij de tweede loslaatproef dezelfde als ontworpen voor de proef van 1971. Naast het in 1971 gebruikte perceel, nu in wintertarwe, is 1 ha uien gezaaid. Vangschermen om een schatting van de samenstelling van de uievliegpopulatie te kunnen maken werden regelmatig verspreid op en om het veld uitgezet. In het begin van de vluchtperiode werden de gesteriliseerde poppen, bestraald als 11 dagen oude poppen (21° C) met 3 kR X-stralen, ingegraven in het tarwevedd, waar ook de wilde poppen zich bevonden. Aangezien de ingraafplaatsen al snel ontdekt en vakkundig geleegd werden door muizen, zijn ze verplaatst naar het open uieveld. Vanaf begin Juli werd iedere week een andere plaats gekozen om het gevaar van infectie van de jonge vliegen door *Entomophthora* te verminderen. Teneinde een betrouwbaar beeld van de verrichtingen van de steriele dieren te krijgen werden allen van een kleurmerk voorzien.

Voor het loslaten van de steriele insecten werd van hetzelfde schema uitgegaan als in 1971. Het begin van de eerste vlucht werd bepaald aan de hand van depots met diapause-poppen, aangelegd in de herfst van 1971. De eerste vlieg verscheen op 30 April, een normaal tijdstip, doch door het koude weer in Mei trad een grote vertraging in het verdere verloop van de uitkomst op. Eind Mei was pas 30% van de vliegen uitgekomen. Begin Juni versnelde het tempo aanzienlijk en op 18 Juni was 95% uit de pop gekomen. Hoogstwaarschijnlijk zijn de wilde vliegen die zich in het tarweperceel bevonden nog later uitgekomen. De bodemtemperatuur was onder de wintertarwe beduidend lager dan bij de depots die op niet begroeid terrein stonden.

De curves die uit de gegevens over de verhouding steriele/fertiele vliegen in de vangsten enerzijds en uit de gegevens over de verhouding steriele/fertiele eieren in de legsels van fertiele wijfjes anderzijds samengesteld kunnen worden blijken gedurende het gehele seizoen goed te correleren. De verminderde competitiviteit van de steriele mannetjes, zoals waargenomen in de tweede helft van 1971, is in 1972 door het ver-

spread loslaten van de vliegen vermeden. Ondanks de behoorlijke mate van infectie, werden de steriele vliegen niet in sterkere mate door de schimmel Entomophthora geïnfecteerd dan de fertiele vliegen. Beide curves volgen duidelijk de loslaatcurve. Dit betekent dat de fertilititeit van de eieren gereguleerd kan worden door het loslaten van grotere of kleinere aantallen steriele vliegen.

Vanuit het gewenste niveau van bestrijding uit gezien, is de reductie van de voortplanting nog niet optimaal geweest. Dit hangt samen met een gebrekkig inzicht in het verloop van de fertiele populatie. Het blijkt dat de verschijningscurve van de populatie, gegeven het beginpunt, moeilijk te voorspellen is, en dat de twee generaties elkaar overlappen. Het loslaatschema voor de proef van 1973 zal met deze ervaringen rekening houden. Tijdens de gehele vluchtperiode van Mei tot begin September zijn in totaal 460.000 gesteriliseerde vliegen uit de wekelijks in het veld gebrachte poppen gekomen. Vlak voor de oogst is het aantal in de grond aanwezige uievliegpoppen geschat op 9000 per ha.

De massakweek (J. Noorlander)

Uit de massakweek schema's van 1971 en 1972 voor het kweken van de poppen die nodig waren voor de veldproeven, is gemakkelijk af te leiden dat het -noodzakelijk hoge- productie niveau te laat in het kweekseizoen werd bereikt. Dit betekende dat er in beide jaren tot midzomer, dus ten tijde van de loslatingen, nog druk gekweekt diende te worden. Steeds is gebleken dat de aanloop van de massakweek langer is dan verwacht wordt. Voor de massakweek van 1972/1973 is het gehele kweekschema vervroegd en wordt getracht de zgn ouder-generaties in een hoger tempo te passeren.

De in 1972 losgelaten vliegen waren van een goede kwaliteit. Tijdens de kweekperiode kwamen ditmaal geen onverwachte schommelingen in de reproductie capaciteit van de vliegen voor. Deze continuïteit wil echter niet zeggen dat de kweekomstandigheden optimaal zijn. De vroege sterfte van vliegen in kweekkooien is een factor die de rationaliteit van het gehele kweekgebeuren duidelijk negatief beïnvloedt. Het gedragsonderzoek (B. Bol) leverde aanwijzingen voor verbetering van de kweekomstandigheden, waaraan wordt voortgewerkt.

In Augustus 1972 werd opnieuw een groep wilde poppen verzameld. De 10.000 poppen grote groep zou via drie laboratoriumgeneraties de basis moeten vormen voor de in 1973 benodigde twee miljoen vliegen. Tijdens het doorkweken naar de eerste generatie trad echter een grote vliegensterfte op, waardoor het schema niet gehandhaafd kon blijven. De oorzaak van de sterfte is niet met zekerheid aan te geven, maar gedacht wordt aan vergiftiging door insecticiden, door PCB uit plastic, e.d. Het blijkt moeilijk een kweek te hebben temidden van afdelingen met anders

gerichte interessen. De tegenslag is opgevangen door inkruisen van de tweede generatie van de in 1971 verzamelde stam.

Radiobiologie en radio-isotopen (J.Ph.W. Noordink)

Om na te gaan of het mogelijk is een radioactief merk in de uievlieg te krijgen dat nog in de volgende generatie aantoonbaar is, werden larven gemerkt door hen medium aan te bieden waaraan 100 uCi ^{65}Zn per 200 gram medium was gemengd. Hoewel de radioactiviteit van de poppen slechts gering was, nl 100-140 tikken per minuut, bleek dit toch voldoende om in vliegen van de volgende generatie na 6 weken belichting autoradiografisch het merk aan te tonen. Momenteel worden proeven gedaan waarbij slechts één van de ouders met ^{65}Zn gemerkt is.

Ten einde een idee te krijgen van de omvang van de fertiele populatie uievliegen op het terrein van de loslaatproef, werden ruim 4000 met ^{65}Zn gemerkte vliegen losgelaten.

Het is nog steeds niet mogelijk gebleken in grote veldkooien met een inhoud van 30 m³ schade in het uienbestand te krijgen, ongeacht het aantal fertiele vliegen dat wordt losgelaten. In deze kooien werden proeven gedaan om de voedselbronnen van de volwassen vliegen te bepalen. Vier soorten voedsel werden aangeboden: honingdauw van de erwtenluis, Acyrtosiphon pisum, op boneplanten, bloemen van een composiet, bloemen van een schermbloemige en het in het laboratorium gebruikte droogvoer. In elk van de vier kooien werd één van deze voedselbronnen met ^{32}P gemerkt. Het bleek dat de honingdauw wordt gegeten, maar dat deze niet voldoende is om de ovariën normaal tot ontwikkeling te laten komen. Noch de composiet, noch de schermbloemige werden in voldoende mate genuttigd om de vliegen aantoonbaar radio-actief te maken. Wanneer, zoals in kleine kooien in een insectarium gebeurde, deze planten als enige voedselbron werden aangeboden, stierven de vliegen binnen enkele dagen. Uit deze reeks van proeven bleek dat het normale droogvoer geredelijk wordt opgenomen en een normale ovariën ontwikkeling ten gevolge heeft. Daar echter dit voedsel in de natuur niet voorkomt, is het nog steeds niet duidelijk welke voedselbronnen de vlieg in het veld gebruikt.

Met J. Theunissen werden nogmaals enkele tientallen vliegen met thymidine ^3H geïnjecteerd om de duur van de spermatogenese vast te stellen. Aanwijzingen werden verkregen dat de duur 8 tot 9 dagen is. Proeven met bestraalde vliegen toonden aan dat de spermatogenese door een bestraling met 3 kR volledig tot stilstand komt.

Daar bij het in ongerede raken van de electronen generator uitgeweken moet worden naar een Röntgenmachine of een ^{60}Co bron, werd nagegaan of de resultaten van bestralingen met deze beide machines overeen kwam met die van de generator. Het bleek dat in noodgevallen zonder bezwaar van de andere faciliteiten gebruik gemaakt kan worden. Voor kleine aantallen te bestralen poppen kan de Röntgenmachine gebruikt worden, terwijl voor zeer grote aantallen poppen het gebruik van de ^{60}Co bron voor de hand ligt.

Proeven om door het aanbieden van stabiele isotopen in het larvale stadium de vliegen en masse te kunnen merken en ze na terugvangst door middel van neutronenaotivering te kunnen herkennen, hebben tot dusverre niet tot het gewenste resultaat geleid. De volgende elementen werden bij deze proeven getest : AuCl_3 , CuSO_4 , Dy_2O_3 , MnSO_4 , AgNO_3 , KBr , NaCl , KCl , ZnCl_2 , en Eu_2O_3 . Begin 1973 zullen nog enkele proeven met verrijkt ^{58}Fe worden gedaan. Blijkbaar heeft het pre-pupale purgeren van de darm tot gevolg dat alle stoffen die niet zijn gemetaboliseerd uit de darm verwijderd worden. De tot dusverre in de literatuur beschreven proeven hebben alleen betrekking op volwassen dieren die via voedsel of water stabiele isotopen opnemen, terwijl het er ons om te doen is het merk gedurende het larvale stadium in te bouwen.

Ecologie van de uievlieg (M. Loosjes)

Er zijn op Flakkee twee veldproeven uitgevoerd waarin de verplaatsingen van losgelaten vliegen werden nagegaan. De eerste proef, tijdens de voorjaarsvlucht, bestreek alle uievelden van 1971 en 1972 binnen een gebied van $1 \times 1\frac{1}{2}$ km. Uit 67.000 in het veld gebrachte poppen zijn door vraat door muizen, sterfte en diapauze-inductie slechts 17.000 gemerkte vliegen gekomen. Met ruim 60 vangschermen werden ten gevolge van het koude voorjaar slechts 71 gemerkte uievliegen teruggevangen, verspreid over een periode van bijna vier maanden. Uit de resultaten van deze proef konden de vliegenpopulaties worden geschat die op drie der 1971-uievelden uitkwamen. Deze bleken ongeveer 2, $2\frac{1}{2}$ en 4 vliegen per m^2 te bedragen, en bleken evenredig te zijn met de geringe, voor uievelden waarin de uievlieg chemisch bestreden is normale, schade in 1971.

Bij toenemende afstand tot het loslaatpunt blijkt de terugvangst per vangscherm, logaritmisch uitgezet, min of meer lineair af te nemen. De punten in deze proef die berusten op vangsten van vliegen die over een 20 m brede kreek zijn gevlogen weken hier niet van af. Zo'n kreek lijkt dus geen barrière voor de zich verspreidende uievliegen te vormen. Het tijdstip van 50% uitkomst van de poppen bleek tot $1\frac{1}{2}$ maand te kunnen verschillen ten gevolge van verschillende boven de poppen aanwezige vegetaties. Dit kan de onduidelijke scheiding tussen de verschillende generaties in het veld verklaren.

In de tweede proef, tijdens de zomervlucht, kwamen uit 40.000 poppen 27.000 gemerkte vliegen. De kwaliteit van deze kleurmerking werd gecontroleerd. Hierbij bleek 100% der vliegen herkenbaar gekleurd te zijn, waarvan bijna 98% niet over het hoofd gezien zou kunnen worden. De vliegsnelheid bleek sterk afhankelijk van de hoek tussen de windrichting en de vliegrichting. Uievliegen kunnen zich met naar schatting maximaal 100 meter per dag verplaatsen met wind pal tegen. De ruim 400 terug-

gevangen vliegen maken het waarschijnlijk mogelijk om het terugvangstenpatroon te toetsen tegen verschillende uitkomsten van een simulatiemodel voor de verplaatsingen van uievliegen. Dit model, ontwikkeld in samenwerking met dr. M. Frissel (ITAL), voorziet desgewenst in : diffusie van de vliegen, preferentie van de vliegen voor bepaalde delen van het proefgebied (bv. uievelden) in de vorm van een lokale vermindering van verplaatsingen, attractie van de vliegen door bepaalde delen van het proefgebied uit alle richtingen of alleen tegen de wind in, en een absolute of relatieve barrière.

De in deze proef teruggevangen wijfjes werden op ovariënontwikkeling en geparrd-zijn gecontroleerd. Via logit-transformatie kon worden bepaald dat het percentage geparrd van 0 tot vrijwel 100 toenam bij wijfjes tussen 7 en 9½ dag oud. De eerste eieren bleken tot ontwikkeling te komen in wijfjes op een leeftijd van 8 tot minder dan 14 dagen.

De gedragswaarnomingen in uieveld zijn voortgezet en uitgewerkt. Uit de gemiddelde opvliegfrequentie van wijfjes van ongeveer eens per 5 minuten, en hun gemiddelde vliegafstand van ruim 20 cm, volgt een in een uieveld afgelegde weg van ongeveer 40 meter per dag van 16 uur. Voor mannetjes ligt deze waarde omtrent 60 meter per dag. In merk-terugvang proeven is geen verschil in verplaatsingssnelheid tussen de sexen gevonden. De langere weg door de mannetjes afgelegde weg zal veroorzaakt zijn door hun typische vliegen van sprongen, waarbij de netto verplaatsing vaak verwaarloosbaar is. Uievliegen vliegen normaal nooit hoger dan de vegetatie ter plaatse reikt, de wijfjes vliegen in een uieveld met een gewashoogte van 30-50 cm gemiddeld op bijna 20 cm hoogte, de mannetjes vliegen hier gemiddeld op ongeveer 30 cm ten gevolge van hun sprongen die tot de toppen der uiebladeren reiken.

Er is een begin gemaakt met het onderzoek naar dagritmen. Uitkomen van de vliegen bleek vooral in de ochtend plaats te vinden, ze vliegen vooral in de namiddag in de vangschermen.

Vleugeladerafwijkingen bleek per populatie in frequentie en type sterk uiteen te kunnen lopen. Een kweekstam had 3% afwijkingen, alleen aan de achterste dwarsader (tp). Op een plaats op Flakkee werd 25% afwijkingen gevonden waarvan meer aan de voorste dwarsader (ta) dan aan de achterste.

Histopathologisch onderzoek (J. Theunissen)

Aan de ontwikkeling van de gonaden van de uievlieg in het popstadium werd veel aandacht besteed. Van de weinige organen waarvan de ontwikkeling van larve tot imago zonder morfologische discontinuïteit plaatsvindt zijn de gonaden het meest geschikt om te dienen als indicator voor een normale ontwikkeling van de pop als geheel. De ontwikkeling van de ovarien en testes in poppen van verschillende ouderdomsklassen is bestudeerd en beschreven. Van vergelijkbare poppen die, 3 dagen oud, bestraald waren met 3 kR X-stralen is de reactie op de bestraling bestudeerd. Typische symptomen, die ook reeds in cytologische preparaten waren gevonden, werden waargenomen. De poppen ontwikkelden zich zeer slecht en een enkele gevormde vlieg was niet in staat de pop te verlaten.

Het autoradiografisch onderzoek van de normale spermatogenese werd afgesloten en aangevuld met een vergelijkbare serie met bestraalde dieren. Bestraling met 3 kR X-stralen blijkt voor de germinale celtypen o.m. te resulteren in een volledige remming van de DNA-synthese, doch niet voor somatische celtypen.

Begonnen werd met het electroenmicroscopisch onderzoek van de spermatogenese. Het eerste doel is een inventarisatie uit te voeren van de verschillende celtypen die bij de spermatogenese zijn betrokken. Dit houdt tevens in dat verband gelegd moet worden tussen de ultrastructuur van deze cellen en hun fysiologische rol en activiteit op grond waarvan zij worden geïdentificeerd. Later zullen de oonsequenties van bestraling voor de ultrastructuur en fysiologische activiteit worden bestudeerd, waarmee een grote stap gezet zal kunnen worden naar de interpretatie van stralingssymptomen en van stralingsgevoeligheid, een der grootste problemen van de stralingsbiologie.

Om te onderzoeken of de resultaten van het onderzoek naar de spermatogenese van de uievlieg en de invloed van ioniserende straling hierop voor een groter aantal belangrijke insecten bruikbaar kunnen zijn, werden van een aantal insecten, die veel worden gebruikt als proefdier en als vertegenwoordigers van verschillende orden, de testes van jonge imagines gefixeerd en verwerkt tot histologische preparaten. Dit geschiedde op de standaard manier zoals die wordt gebruikt voor de uievlieg.

Onderzoek uitgevoerd door derden

Bij het zoeken naar lokstoffen voor de uievlieg, een onderwerp dat was aangesneden door het Centraal Laboratorium TNO, Delft (dr. W. Wientjes), bleek meer kennis van het gedrag van de vliegen noodzakelijk om een zinvolle toetsmethode te kunnen ontwerpen. De heer B. Bol (student R.U., Leiden) heeft dit moeilijke onderwerp aangepakt. De heer W. Kelderman (student L.H., Wageningen) rondde zijn onderzoek over diapauzeinductie en -doorbreking af. Onderzoek over verbetering van de kweekmethoden en over de ontwikkelingssnelheid van poppen ivm de verschijning van de vliegen in het voorjaar werd begonnen door de heer R. Houwing (student L.H., Wageningen). Een kort verslag over de twee eerste onderzoekingen volgt hieronder.

Paringsgedrag (B. Bol)

In laboratoriumonderzoek is het paringsgedrag geanalyseerd. Inleiding tot een paring is het beklimmen waarbij een mannetje nauwelijks onderscheid maakt tussen de geslachten. Dit onderscheid vindt pas plaats na dit treffen van twee dieren, waarbij de factoren die acceptatie of afstoten van de partner bepalen niet geanalyseerd konden worden. Stra-

lingswarmte afkomstig van de zon of van waratelampen leidde onder de proefomstandigheden verhoogde activiteit en copulatiepogingen na het ophouden van de instraling in. Het geheel van de activiteiten nam met de leeftijd toe van de 4e tot de 12e dag. De eerste copulatie werd op een leeftijd van 7 dagen waargenomen. De loop- en vliegactiviteit van zowel mannetjes als wijfjes nam toe bij aanwezigheid van uiegeur evenals het beklimmen door de mannetjes. Waarschijnlijk bevordert de geur van wijfjes de loop- en vliegactiviteit van de mannetjes.

Verbreking van de diapauze (W. Kelderman)

Het verbreken van diapauze blijkt een temperatuur afhankelijk proces dat zich bij lagere temperaturen veel sneller afspeelt dan bij hogere. In de proefopzet was inbegrepen een bewaarduur van 0, 5, 10, 15 en 20 weken en bewaartemperaturen van 3, 6, 10, 15 en 20°C. Na de bewaring werden de poppen bij 22°C gebracht en het percentage en het tijdstip van uitkomen bepaald. Het meest effectief voor het verbreken van de diapauze waren de lage temperaturen van 3 en 6°C. Een bewaarduur tussen 5 en 10 weken is voldoende om de diapauze in bijna alle poppen op te heffen. Bij 10°C is hiervoor een langere bewaring nodig, nl tussen 10 en 15 weken. Bij 15 en 20°C wordt de diapauze niet geheel opgeheven. De langzame verbreking gaat hier gepaard met een hogere popmortaliteit.

Gezien de bewaarduren die nodig zijn bij 3, 6 en 10°C om de diapauze op te heffen, mag worden aangenomen dat in het veld alle diapauzepoppen in Januari geactiveerd zijn. Het verschijnen der eerste vliegen is dan afhankelijk van de temperatuursom boven de ontwikkelingsdrempel. Dit aspect wordt nader bestudeerd (R. Houwing).

Associato della Commissione: Comitato Nazionale
per l'Energia Nucleare, Laboratorio Applicazioni Agricoltura
N° del contratto: 107-72-1 BIOD

Capo del gruppo di ricerca: prof. A. Bozzini

Tema generale del contratto: Application of sterile-male
technique for insect control

-
- a. Pre-releasing phase. With the ecological data obtained in the island of Procida in previous years it was ascertained that, during the autumn-winter period, the medfly, besides passing its pupal stage in the soil and its adult stage in this environment, could hire its larval and pupal stage in the fruit of the sour orange tree, which is a troublesome hotspot of infestation. Therefore, to begin the program under the best possible conditions, the following steps were taken 5 to 6 months prior to the distribution of the sterile adult insects:
- massive and continuous capturing of the male Ceratitis population by means of 5000 "stick" traps using trimedlure. These were placed throughout the entire island to avoid fertile mating and the subsequent over-wintering of the gravid female (quantitatively very difficult to estimate).
 - collection and destruction of 90% of sour oranges.
- b. Mass rearing and pupal handling. From the early part of 1972 Ceratitis breeding in the insectary of Casaccia gradually increased until 4 to 5 million insects were available weekly for the control experiment. The rearing technique was the following: rearing of adult flies in prismatic cloth cage-, 125 cm x 80 cm x 40 cm, each cage containing 50,000 adults;

larval rearing in "popping" diet; synchronization of the length of the pupal period by means of temperature varying from 15° to 25°C. Three days before the end of the pupal stage the pupae were first suitably colored with fluorescent dyes and placed in special paper bags for releasing, in quantities of not more than 10,000 insects per bag. They were kept in air-conditioned cells at 25°C until the time for their irradiation.

c. Radiation and transport. The paper bags containing emerged adult flies were directly irradiated in the gamma irradiation plant of Agricultural Applications Laboratory of CNEN's Casaccia Center, the dose varying from 9.2 to 13.0 Krads. The insects were transported to Procida with CNEN's car and by ferry. The release of the adult sterile insects was carried out directly by the farmers who received a certain number of bags, in proportion to the extension and importance of their area cultivated by fruit. In areas which were not easily accessible, releasing of the insects was carried out by the CNEN personnel. Normally there were 2 shipments per week, for a total of approximately 600 bags released; each shipment affecting alternately half of the total surface area of the island (table I).

d. Methods for evaluating the effectiveness of the technique
To evaluate the effectiveness of the control method used, the same control technique were used as in the 1969 experiment at Procida. In each of the 35 sampling areas chosen in the island the following controls were made weekly: examination of fruit to check for infestation; capturing adult flies with "Nadel" type traps activated with Trimedlure and determination of egg hatch taken from insect oviposition on peaches. In the control areas of Mount of Procida, Ischia and Capri, the same controls were made every 7 - 15 days with the exception of examining the puncture site, which was done only on Mount of Procida.

Risultati del progetto N. 1

Capo del progetto: Ugo Cirio e Italo De Murtas

Titolo del progetto: Biological control of med-fly by sterile insect technique in the island of Procida

PRINCIPAL RESULTS OF RESEARCH

a. Fruit infestation

Control of infestation was made on the main plant hosts of the Ceratitis present in the various sampling areas. This was done by picking at random an amount of fruit from the trees and placing them in suitable boxes, and by visually examining the fruit hanging on trees and those which had fallen to the ground. In table III, it can be observed that in the month of March, when the sterile adults were first released, only approximately 4% of the sour oranges were attacked. There was no indication of infestation until end-August, notwithstanding the fact that thousands of fruit were sampled and that control was extended to zones other than the sampling zone. Viceversa, in the control areas, the infestation began in the month of June and continued to spread, until practically all the fruit was destroyed at end-August, in spite of the fact that the farmers has used phosphoric-ester insecticides to limit infestation. Towards the end of August in Procida, there was a slight unexpected infestation of peaches which could not be subsequently eliminated, notwithstanding the presence of a good number of sterile adults. However, this infestation which mainly attacked peaches and figs was slow-moving and never reached the disastrous levels which were observed

in the control areas. It has been proven, however, that the island of Procida has never been completely isolated from the mainland on account of a continuous immigration of wild flies to the island (fig. 1), which previous studies on the movement of the species effectuated in 1968-1969 never clarified. It is likely that this immigration is composed of already fertilized females who would not be receptive nor would mate with the sterile adults present. This immigration begins at end-August which coincides with the fruit infestation. To confirm this theory could be the fact that emerged adults from this infested fruit do not behave as laboratory flies in their capacity to deposit eggs in cloth cages.

b. Trapping

In table I the proceedings of the trapping which took place at Procida can be observed. 140 traps were exposed weekly for 24 hours in the various sampling areas. Note that wild males of Ceratitis were captured only at the beginning of the experiment and again at the beginning of the end of August.

These latter capture would coincide with the beginning of the island's August infestation and almost certainly with the initiation of the immigration of the* species. Nevertheless, even after the month of August, the number of wild flies captured never exceeded more than one fly per day and per trap. On the contrary, in the control areas the number of flies captured was also more than 12 per day and per trap (fig; 2).

In the control areas, the proceedings of the trapping was very different, which was probably due to the different environmental conditions in which the species developed. While the population of Ceratitis increased rapidly in Capri, it was very slow in Ischia. An indirect evaluation of the effectiveness of the control technique was made by comparing the population growth of Ceratitis in Procida during the years 1968, 1969 and 1972 (fig. 3). From the graph we can see that in 1968 the wild adult flies reproduced rapidly beginning at mid-July until mid-August. In 1969, they were in great quantity already in the month of June, but they remained within the economic injury population level for the entire releasing period, though they later did reach the same population density as the preceding year.

In 1972, the number of adult flies captured was almost irrelevant, with an increase only during the month of October, which is directly correlated with the immigration movement of the wild flies. Here, however, from the trapping, it seems clear that this number is not so great as to cause enormous hotspots of infestation. From the same figure, one can observe the population growth in relation to the density of sterile insects per hectare. In the 1972 experiment, it is noted that the high number of insects released at the beginning of the experiment had completely eradicated the local fly population from the month of June until end-August. In the 1969 experiment, due to the late release and low number of insects released, the population of the island species was not suppressed, not even for a short period.

It is evident, however, that the trapping system of capturing adult flies is not a suitable method of control. The reasons being the difficulty of recapturing a sufficient number of the adult flies released to enable an accurate estimation of the population, and the different behaviour the breded flies and wild flies have towards trimedlure

c. Examination of the puncture site of oviposition

This control finally demonstrated that on the Island of Procida, the infestation present during the month of August and the following months was at a very low level. In fact, checking the puncture sites (table III) it was found that only 5.7% of the puncture sites in Procida had eggs, in comparison with 100% at Mount of Procida. In regard to the egg hatch, collected and placed in petri dishes, there was no difference between the two areas of control during the month of August. It is possible, therefore, that at Procida, the eggs collected were deposited by females which had immigrated and did not mate with sterile adults. In the month of September, while the egg hatch count was high ⁱⁿ Mount of Procida (data collected by dott. Fimiani on persimmons,) in the release area of Procida it was only 15.5%. This difference was probably due to the contemporary presence of sterile insects, newly-emerged insects and insects which had immigrated. The fact remains, however, that the adult sterile insects do have a definite effect on controlling the fertility of adult wild flies.

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- Cirio, U. and I.D. De Murtas. Status of Mediterranean fruit fly by the sterile male technique on the island of Procida. IAEA/FAO Panel 1972 (in press).
- De Murtas, I.D. and U. Cirio, 1972. Induzione di sterilità nella mosca mediterranea della frutta (Ceratitidis capitata Wied?) con raggi gamma e neutroni. IX Congresso Italiano di Entomologia, Siena, Giugno 1972.
- De Murtas, I.D. and U. Cirio, 1972. L'allevamento massivo della mosca della frutta Ceratitidis capitata Wied. nell'inset-tatio della Casaccia ed aspetti economici della lotta autocida. IX Congresso Italiano di Entomologia, Siena, giugno 1972.

TABLE I - DATA ON RELEASED AND RECAPTURE OF HONEYFLY ON THE ISLAND OF PROCIDA, ITALY. (1972)
(1) TAKEN IN THE FIELD 72 HOURS-AFTER RELEASE.

DATA OF ARRIVAL	WEEKLY SHIPMENTS	No. PIPAE PER WEEK (millions)	PERCENT OF EMERGENCE (1)	ADULTS RELEASED (millions)	NUMBER OF ADULT BAGS	No. MALES CAPTURED		RATIO STERILE/WILD
						STERILE	WILD	
MARCH 24	1	1.000	91.3	.931	235			
2P	1	1.250	99.5	.743	250			
APRIL 7	1	1.700	21.7	.368	350	632	2	316 : 1
11	1	1.500	50.9	.764	300	2	0	
1P-21	2	3.075	75.8	2.330	615			
24-2P	2	3.125	44.9	1.404	625	418	4	104 : 1
MAY 5	1	1.550	89.2	1.382	310	64	1	64 : 1
9-12	2	3.150	94.1	2.965	630	507	0	
16-19	2	3.400	88.7	3.01P	680	403	0	
23-26	2	4.775	87.4	4.173	635	1199	0	
30	1	1.500	82.4	1.236	300	2958	0	
JUNE 6-9	2	5.540	83.3	4.613	615	6762	1	6762 : 1
13-16	2	5.600	70.0	3.923	610	1991	1	1991 : 1
20-23	2	5.600	76.0	4.258	620	2653	0	
27 and July 1	2	6.200	91.6	5.681	620	2233	0	
JULY 4	2	6.100	75.5	4.608	620	3240	0	
11-14	2	4.700	82.9	3.897	597	1007	0	
18-21	2	4.500	74.3	3.345	640	2727	0	
25-28	2	5.700	79.6	4.141	655	1775	0	
AUGUST 1-4	2	3P00	66.3	2.522	610	2183	0	
8-11	2	4.400	51.2	2.255	635	1795	0	
17	1	2.700	47.1	1.271	290	306	0	
22-25	2	5.300	47.5	2.520	660	662	0	
29 and Spt. 1	2	4.200	57.1	2.399	660	2452	12	204 : 1
SEPT. 6-8	2	3.100	50.7	1.572	620	1923	2	961 : 1
12-15	2	5.400	72.8	3.933	790	1851	0	
19-22	2	6.100	52.9	3.231	800	2986	2	1493 : 1
26-29	2	5.200	48.2	2.509	780	3140	3	1046 : 1
OCTOBR. 3-6	2	3.650	50.1	1.830	620	3070	0	
10-16	2	5.900	60.7	3.582	590	2294	9	254 : 1
18-20	2	5.400	31.2	1.685	686	1359	38	88 : 1
24-28	2	4.100	57.1	2.342	650	650	30	21 : 1
TOTAL	57	128.715	̄ 64.3	85.413	16.398	55.242	105	

TABLE 11 - NUMBER OF DIFFERENT HOST FRUIT EXAMINED AND PERCENTAGE ATTACKED BY MEXFLAY IN THE RELEASE AND CONTROL AREAS (1972)

A = TOTAL FRUIT EXAMINED ; B = % ATTACKED ; * = DATA COLLECTED BY DR. FINIARI (ISTITUTO ENTOMOLOGIA AGRARIA-UNIVERSITA' DI NAPOLI-PORTICI)

(-) = No attack (/) = No fruit available.

DATE	LOCALITY																									
	PROCIDA						MONTE DI PROCIDA *						CAPRI						ISCHIA							
	ORANGES		ORANGES		PEACHES		APRICOTS		PEARS		FIGS		PEACHES		APRICOTS		PEARS		FIGS		PEACHES					
	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B	A	B				
30/III	16120	0.3	1572	4.1																						
15/IV	9200	-	3164	1.3																						
30/IV	4796	-	927	0.6																						
15/V	340	-	675	-	468	-	744	-				/	/		/	/					/	/				
30/V	700	-	156	-	2629	-	3823	-				/	/		/	/					/	/				
15/VI	1490	-	381	-	8280	-	6175	-	450	-	5054	-	/	/		/	/		/	/	/	/				
30/VI	344	-	36	-	6483	-	147	-	610	-	/	/	100	20.0	/	76	6.6	100	25.0	/	/	/	/			
15/VII	/		12	-	3773	-			997	-	/	/	132	19.7	290	30.7	80	2.5	115	52.2	1505	72.9	375	6.6	/	/
30/VII	/		/		4609	-			1892	-	/	/	45	64.4	6	100.0	20	30.0	200	76.0	/	/	205	25.0	/	/
15/VIII	/		/		72648	-			750	-	4050	-	5	80.0	-	-	203	47.7	315	79.1	/	/	/	/	975	11.6
30/VIII	/		/		15889	0.6			575	-	8930	-	/	/					1392	79.9	/	/	805	62.2	1199	31.5
15/IX	/		/		2245	0.6			418	-	4635	-							1685	72.7	/	/	614	71.8	457	90.0
30/IX	400	-	500	-	1238	12.0			350	-	4251	1.1							1172	88.2			175	80.6	/	/
15/X	26180	0.6	610	-	181	11.1			100	-	415	-							736	93.2			340	70.0	/	/
30/X	23270	0.004	1020	-					/	/									/	/			/	/		

TABLE III—DATA ON PUNCTURES AND EGGS FERTILITY OF PEACHES EXAMINED IN THE RELEASE AND CONTROL AREAS. (PUNCTURES SELECTED FOR STUDY NON-RANDOM SAMPLE)

*Data collected by Dr. PIMIANI - Istituto Entomologia Agraria - Portici - NAPOLI -

CRITERIA	LOCALITY				BACOLI [•]
	PROCIDA				
	30/VI	30/VII	30/VIII	30/IX	30/VII
NO OF FRUIT ANALYZED	180	327	402	169	37
TOTAL No. PUNCTURES (a+b+c)	117	320	261	220	181
MEAN PUNCTURES/FRUIT	0.65	0.98	0.65	1.3	4.9
PUNCTURES WITHOUT EGGS (a)	112	310	246	182	0
PUNCTURES WITH EGGSHELLS (b)	0	0	4	9	137
PUNCTURES WITH EGGS (c)	5	10	11	35	44
PUNCTURES (b+c) (%)	0	0	5.7	20.0	100
EGGS COLLECTED	35	119	72	187	168
EGGS HATCHED (%)	0	0	90.0	15.6	88

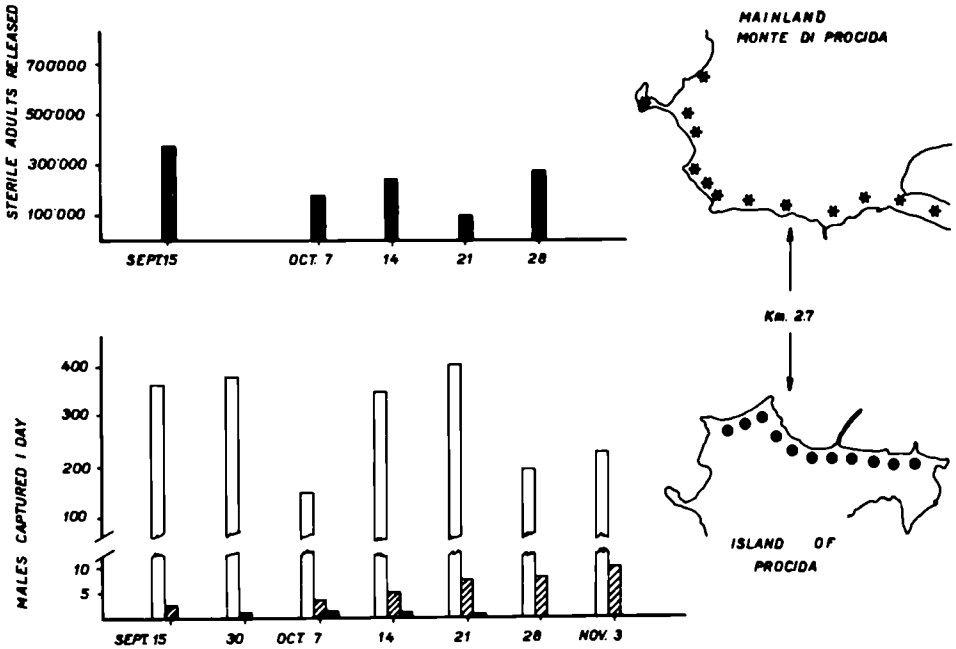


FIG. 1 - NUMBER OF MEDFLY CAPTURED ALONG THE NORTH COAST OF PROCIDA IN RELATION OF STERILE ADULTS RELEASED ON MONTE DI PROCIDA
 □ - MARKED OF PROCIDA ▨ - WILDS ■ - MARKED OF MONTE DI PROCIDA
 * - RELEASE POINTS ● - TRAP POSITION (TOTAL 40 TRAPS DAILY CONTROLLED)

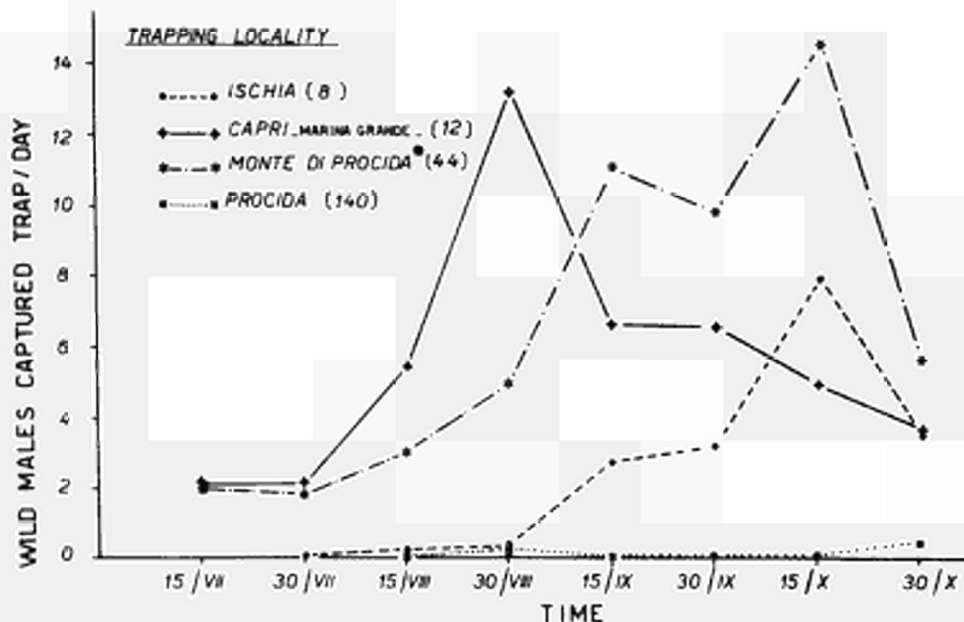


FIG. 2. NUMBER OF CAPTURED WILD MALES ON PROCIDA AND CONTROL AREAS (1972).
No. OF TRAPS () PER AREA.

● DATA FROM Dr. FIMIANI, ISTITUTO ENTOMOLOGIA AGRARIA, PORTICI, NAPOLI.

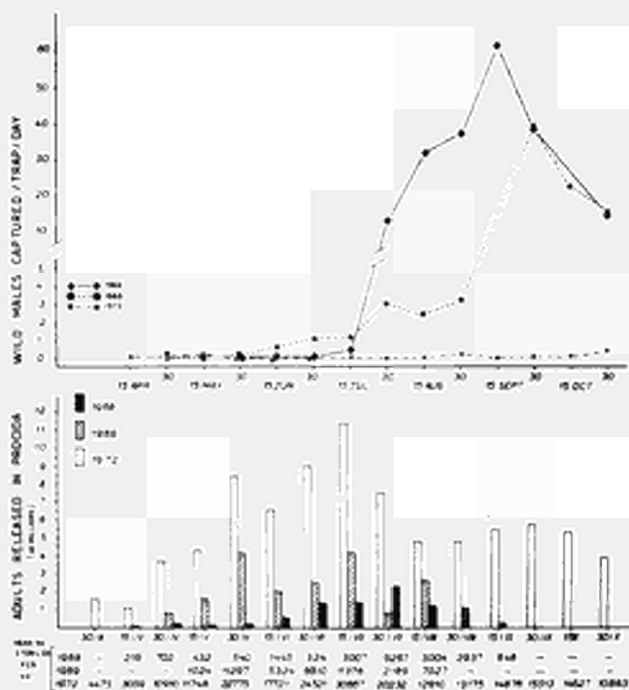


FIG. 3. RELATIONSHIP BETWEEN NUMBER OF WILD MALES CAPTURED AND NUMBER OF STERILE MEDFLY RELEASED DURING THE EXPERIMENTS IN PROCIDA (ITALY).

NUKLEARMEDIZIN

NUCLEAR MEDICINE

MEDECINE NUCLEAIRE

Vertragspartner der Kommission: Universität Ulm,
Sektion Nuklearmedizin

Nr. des Vertrags: 116 - 72 - 1 bio d

Leiter der Forschungsgruppe: Doz.Dr.W.E.Adam

Allgemeines Thema des Vertrags: Entwicklung eines
nicht traumatisierenden Verfahrens zur Analyse der
Herzkinetik mit Hilfe der quantitativen Funktions-
szintigrafie und Anwendung simulierender Verfahren.

Im Jahre 1972 konnten lediglich Vorarbeiten zum For-
schungsvorhabens geleistet werden, da die zur Durch-
führung der Arbeit notwendige Platte noch nicht ge-
liefert werden konnte. Um die dadurch aufgetretenen
Schwierigkeiten zu umgehen, wurden Programme ent-
wickelt, die das zweidimensionale signal averaging mit
der Apparatur ermöglicht, die in der Ulmer Nuklear-
medizin bereits vorhanden ist.

2 Programme wurden entwickelt:

- 1) Indirektes zweidimensionales Averaging (IND-AV)
- 2) Direktes zweidimensionales Averaging (DIR-AV)

Beim direkten Averaging werden Bilder mit je 1000
Speicherplätzen von je 40 msec Aufnahmezeit phasenrichtig
im Kernspeicher addiert. Bei der Größe des Kernspeichers
(16 K) können 15 Bilder gespeichert werden.

Beim indirekten Averaging werden 1 K Bilder zusammen mit
dem EKG auf Band gespeichert und nach der Untersuchung
phasenrichtig addiert.

Ergebnisse des Projekts Nr. 1

Leiter des Projekts und wissenschaftliche Mitarbeiter:
Doz. Dr. W.E. Adam, Dipl. Phys. F. Bitter,
Ing. grad. J. Olbrich, Dr. E. Sigmund und
Ing. grad. R. Weller

Titel des Projekts: Entwicklung eines nicht traumatisierenden Verfahrens zur Analyse der Herzkinetik mit Hilfe der quantitativen Funktionsszintigrafie und Anwendung simulierender Verfahren.

Die Programme wurden für die alte Konfiguration unseres Rechners erstellt, um erste Erfahrungen sammeln zu können. Aus ersten Testuntersuchungen lassen sich folgende Aussagen machen:

- 1) Das Verfahren ist durchführbar. Nach Injektion eines kurzlebigen Nuklides und Erreichen des Steady State lassen sich die Schwankungen der praecordial erfaßten Impulse als Funktion des Füllungszustandes des Herzens erfassen und in Form einer Zeit- Aktivitätskurve ausdrücken, die die Füllungsveränderungen des Herzens global während einer Herzrevolution beschreibt.
- 2) Das gleiche ist möglich für Teilbereiche des Herzens, d.h. für die linke bzw. rechte Herzhälfte.
- 3) Die geometrische Auflösung ist begrenzt durch das jetzt vorhandene System auf 1000 Speicherplätze/Bild (5x5 bit).
- 4) Die zeitliche Auflösung beträgt 40 msec. Beim direkten Averaging kann eine günstigere zeitliche Auflösung im allgemeinen lediglich durch den Verzicht auf die vollständige Erfassung der Herzaktion erlangt werden.

Mit der 1973 erwarteten Platte lassen sich die unter 3) und 4) genannten Schwierigkeiten beheben, insbesondere kann die geometrische Auflösung auf 6 Bit gesteigert werden. Die 1972 durchgeführten Voruntersuchungen erlauben jedenfalls die Aussage, daß grundsätzliche Schwierigkeiten technologischer Natur bei der Durchführung des Projektes nicht zu erwarten sind.

Veröffentlichungen:

Da ein unterzeichneter Vertrag bis Ende 1972 nicht vorlag, konnten Veröffentlichungen unter dem Euratomkontrakt nicht erfolgen. Die angesprochene Problematik wurde auf dem IAEA Kongreß Monte Carlo 1972 erörtert:

F. BITTER and W.E. ADAM

A DATA ACQUISITION AND PROCESSING SYSTEM FOR RAPID
DYNAMIC INVESTIGATION WITH A SCINTILLATIONS CAMERA

IAEA / SM - 164 / 166, in print

Associato della Commissione:

Centro di Medicina Nucleare Università di Pisa

N. del contratto: 110-72-1 BIOI

Capo del gruppo di ricerca: Prof. Luigi DONATO

Tema generale del contratto:

Ricerca e sviluppo di metodi diagnostici e indagine fisiopatologica mediante l'uso di traccianti radioattivi in vivo ed in vitro.

L'indirizzo fondamentale e comune ai vari progetti del contratto è rappresentato dallo sviluppo di nuovi mezzi di diagnosi precoce con traccianti radioattivi, sia in vitro (radiochimica clinica), che in vivo (medicina nucleare).

Le ricerche di radiochimica clinica si sono rivolte al settore degli ormoni proteici e steroidei, con una particolare enfasi sugli aspetti immunochimici del radioimmunoassay, sulla identificazione e la caratterizzazione di anticorpi anti-insulina presenti in diabetici, ed infine sul lo sviluppo di nuove e più specifiche tecniche di frazionamento e purificazione proteica.

Le ricerche di medicina nucleare si sono sviluppate in tre direzioni: diagnostica polmonare, diagnostica renale e diagnostica coronarica. Tutte sono state caratterizzate dalla integrazione tra approccio morfologico e approccio funzionale, mediante scinticamera, scansioni o contatori multipli. Per lo studio polmonare si è compiuto il lavoro di ca ratterizzazione delle prestazioni della scinticamera in rapporto alle modalità di misura, e nell'ambito renale si è sviluppato un nuovo metodo di indagine separata della funzione dei due reni. Infine nel settore coronarico si è messa a punto una tecnica di studio morfo-funzionale della perfusione regionale del miocardio, mediante impiego di una scinticamera collegata ad un calcolatore di processo, tecnica che ha già fornito risultati originali ed importanti per la comprensione dei meccanismi che intervengono nell'angina pectoris.

Elenco delle pubblicazioni scientifiche

- R. Malvano, G. C. Zucchelli, U. Rosa, A. Salvetti: Measurement of plasma renin activity by angiotensin I radioimmunoassay: (1) an assessment of some methodological aspects.
J. Nucl. Biol. Med. 16, 24, 1972.
- R. Navalesi, A. Pilo, S. Lenzi, P. Cecchetti, G. Corsini, L. Donato: Insulin kinetics in normal and diabetic subjects.
8 Annual Meeting Europ. Assoc. Study Diabetes, Madrid, September 6, 1972.
- C. A. Rossi, Annali Sclavo, 1972 (in corso di stampa)
- C. A. Rossi, A. Lucacchini, U. Montali, Abstr. Comm. 8th FEBS Meeting, Amsterdam, 1972, n. 796.
- C. A. Rossi, A. Lucacchini, G. Ronca, Proc. 2nd AIC-SIB Meeting, Pavia, 1972, n. 4.
- A. Lucacchini, U. Montali, C. A. Rossi, Atti Congresso SIBS, Roma, 1972, n. 160.
- A. Lucacchini, C. A. Rossi, Inter. J. Prot. Research, 4, 1972.
- A. Lucacchini, C. A. Rossi, Inter. J. Prot. Research (in press).
- C. Giuntini, F. Fazio, A. M. Santolicandro, G. C. Strata: Le tecniche scintigrafiche nello studio della distribuzione del flusso di sangue polmonare.
XIII Congresso Nazionale Soc. Ital. Biol. Med. Nucl., Pisa, 3 novembre 1972.
- A. Maseri, P. Mancini: The evaluation of regional myocardial perfusion in man by a scintillation camera computer system.
In: "Myocardial Blood Flow in Man" edited by A. Maseri, pag. 220, 1972.
- C. Bianchi: Measurement of the glomerular filtration rate. Capitolo dell'Opera "Evaluation of kidney function and diseases with radioisotopes", M. D. Blafox Editor, S. Karger Publisher, 1972, pp. 21-53.
- C. Bianchi, A. Coli, R. Palla, P. Giannotti: Sulla diagnosi funzionale ed etiologica mediante conteggio esterno delle nefropatie monolaterali con ipertensione arteriosa.
Atti del III Congresso dell'Associazione Nazionale Medici Cardiologi Ospedalieri, Firenze, marzo 1972 (in stampa).
- C. Bianchi, A. Coli, R. Palla (con la collaborazione di A. Lo Moro): The reliability of ^{140}La -DTPA for the determination of glomerular filtration rate in man.
Atti del X Congresso della Gesellschaft für Nuclearmedizin, Freiburg, Settembre 1972 (in stampa).

C. Bianchi, A. Coli, R. Palla, P. Giannotti: The functional and aetiological diagnosis of unilateral hypertensive kidney diseases using external counting.

Volume degli Abstracts del V Congresso della International Society of Nephrology, Città del Messico, ottobre 1972.

Note amministrative:

Il Prof. Renzo Navalesi ha assunto la responsabilità del progetto n. 2 al quale continua a collaborare il Prof. Umberto Rosa.

Risultati del progetto n. 1

Capo del progetto e collaboratori scientifici:

Prof. Umberto Rosa, Dr. Renzo Malvano, Dr. Carlo Zucchelli, Dr. Cesare Baccini, Dr. Giuseppina Toni

Titolo del progetto: Studio delle caratteristiche cinetiche di antigeni ed anticorpi patogeni per l'uomo e del loro comportamento dopo adsorbimento in fase solida e in condizioni dinamiche.

Studio della cinetica della reazione antigene-anticorpo al fine del dosaggio radioimmunologico dell'angiotensina I e dell'aldosterone.

1. Una tecnica di dosaggio della attività reninica nel plasma (P. R. A.) mediante misura radioimmunologica dell'angiotensina I è stata studiata al fine di una sua ottimizzazione e standardizzazione.

I criteri di standardizzazione seguiti riguardano in particolare il controllo della reazione enzimatica precedente la misura radioimmunologica e le condizioni della reazione antigene-anticorpo.

Per la reazione enzimatica è stata studiata la cinetica di generazione ed il controllo della degradazione dell'angiotensina I da parte del Converting Enzyme e delle angiotensinasi. Per la ottimizzazione della parte ra radioimmunologica del dosaggio si è valutata la sensibilità e la specificità derivante dall'impiego di dieci antisieri differenti per la costante di affinità (K), per il numero dei siti anticorpali (Abo) e per il grado di eterogeneità (α).

2. La determinazione dell'aldosterone plasmatico mediante radioimmunoassay richiede una preventiva separazione e purificazione dello steroide dagli altri steroidi interferenti quali il cortisone ed il cortisolo e a questo scopo si sono studiate le varie tecniche di estrazione e separazione cromatografica assumendo quali criteri di scelta tra i vari procedimenti la percentuale di recupero ed il livello di "Blank".

I risultati conseguiti hanno permesso di misurare l'attività reninica con

maggior accuratezza, precisione e sensibilità e quindi il metodo si è rivelato un ottimo strumento per lo studio e la differenziazione dei vari tipi di ipertensione arteriosa. Per l'aldosterone l'esperienza compiuta ha permesso l'inizio della determinazione radioimmunologica in parallelo al dosaggio dell'attività reninica e l'acquisizione e l'approfondimento delle delicate tecniche di separazione e purificazione di sostanze strutturalmente molto simili.

3. Allo scopo di una ulteriore ottimizzazione del dosaggio radioimmunologico si è iniziato uno studio volto alla separazione di gamma-globuline ed immunoglobuline specifiche. Per questo sono state messe a punto tecniche di elettrocromatografia su colonne di Sephadex e di "affinity chromatography" usando antigeni in fase solida (cellulosa, sepharose).

Risultati del progetto n. 2

Capo del progetto e collaboratori scientifici:

Prof. Renzo Navalesi, Prof. Umberto Rosa, Dr. Silvia Lenzi

Titolo del progetto: Studio delle caratteristiche cinetiche di antigeni ed anticorpi patogeni per l'uomo, e del loro comportamento dopo adsorbimento in fase solida e in condizioni dinamiche.

Studio della cinetica di reazione tra insulina marcata e anticorpi anti-insulina

Sono stati studiati n. 14 soggetti diabetici insulino-dipendenti per la presenza di anticorpi anti-insulina, confrontandoli con n. 9 soggetti diabetici insulino indipendenti (mai trattati con insulina) nel cui siero era dimostrabile la presenza di anticorpi anti-insulina e con un gruppo di n. 13 soggetti normali.

Lo studio ha riguardato:

- a) la messa a punto di metodiche per la determinazione delle immunoglobuline seriche leganti l'insulina e la misura della loro capacità a fissare l'ormone;
- b) la valutazione critica del dosaggio radioimmunologico dell'insulina in presenza di anticorpi (metodi della Heding);
- c) le caratteristiche del tracciante al fine di valutare in vivo la cinetica dell'insulina in presenza ed in assenza di anticorpi.

Per il primo punto (rivelazione e quantizzazione del potere legante di anticorpi anti-insulina) si è studiata l'influenza esercitata in vivo dalla presenza di anticorpi sulla cinetica dell'ormone e la sua utilizzazione metabolica. Si è dimostrato che la presenza di anticorpi anti-insulina determina una marcata riduzione della velocità di distribuzione ed utilizzazione dell'ormone in vivo.

I dati del dosaggio dell'insulinemia in presenza di anticorpi sono risul-

tati allo stato attuale della messa a punto della metodica, utili per una valutazione indicativa del pool insulinico nei pazienti diabetici insulino dipendenti.

Infine per il terzo punto si è rilevata l'estrema importanza di disporre di insulina mono-iodata al fine di una corretta caratterizzazione della cinetica, e conseguentemente la necessità di ricorrere a tecniche di frazionamento cromatografico delle preparazioni traccianti, allo scopo di poter impiegare preparazioni con meno del 2% di molecole diiodate.

Risultati del progetto n. 3

Capo del progetto e collaboratori scientifici:

Prof. Carlo Alfonso Rossi, Prof. Giovanni Ronca, Dr. Antonio Lucacchini, Dr. Umberto Montali

Titolo del progetto: Sviluppo della cromatografia per affinità nel campo delle tecniche traccianti e in chimica clinica.

Messa a punto generale delle metodiche di cromatografia per affinità in vista della loro applicazione a traccianti radioattivi.

Il problema cruciale della preparazione di traccianti per modificazione chimica di proteine pure è risolto in linea di principio dalla cromatografia di affinità. Questa infatti essendo fondata sulle specifiche proprietà funzionali delle macromolecole biologiche permette di separare i "traccianti" autentici funzionalmente attivi, fra le nuove proteine risultanti dalle modificazioni chimiche specifiche necessarie alla marcatura.

Per la messa a punto di questa tecnica si è scelta l'As. deaminasi, enzima da noi ben conosciuto.

Ligande - Sono stati sintetizzati la 9-p-aminobenziladenina e la 9-p-acetamidobenziladenina, analoghi del substrato ed inibitori competitivi dell'enzima, con $K_1 = 4, 5$ e $4, 7 \mu M$, ed è stata misurata la stechiometria della reazione con la proteina preparando la 9-p-bromoacetamidobenziladenina che si lega all'enzima con stechiometria 1:1. Anche questo reattivo alchilante specifico è inibitore competitivo; l'enzima è protetto dall'inattivazione dalla purina riboside (inib. comp. $K_i = 7 \mu M$) ed inoltre si è dimostrato che il reattivo alchilante si lega ad un residuo di lisina essenziale per l'attività.

Si è scelto questo analogo del substrato per preparare l'adsorbente specifico perchè oltre all'alta affinità per il sito dell'enzima, presenta completa stabilità sia all'azione dell'enzima sia a quella delle nucleosidasi (che possono essere presenti nell'estratto grezzo).

Preparazione dell'adsorbente specifico - Il metodo di preparazione consiste nelle fasi seguenti: attivazione del Sepharose con BrCN, copulazione

con 3,3'-diaminodipropilamina, trattamento con anidride succinica ed infine unione della 9-p-aminobenziladenina al Sepharose in presenza di una carbodiimide idrosolubile. In questa fase è stato tra l'altro messo un metodo derivato dalla tecnica di Bratton e Marshall per il dosaggio del numero di residui di inibitori per unità di volume di resina, al fine di standardizzare le preparazioni e migliorare le rese.

Messa a punto dell'eluzione - L'elevata affinità dell'As. deaminasi per l'adsorbente specifico da noi preparato permette l'adsorbimento dell'enzima da estratti grezzi anche molto diluiti ed il lavaggio del complesso con soluzioni saline diluite (fino a 0,1 M in tampone fosfato a pH 7) per allontanare altre proteine. Uno studio comparativo degli eluenti ha condotto all'adozione di soluzioni da 4 a 8 uM di guanilurea (inibitore competitivo dell'As. deaminasi) in tampone fosfato 0,1 M pH 7, 8.

In questo modo è stato possibile purificare in una sola tappa l'enzima da estratti grezzi di mucosa intestinale e di milza di vitello, di duodeno di pollo e di altre sorgenti con ricuperi del 90-100% ed attività specifiche costantemente più alte di quelle riportate in letteratura.

Il metodo si è quindi rivelato un metodo generale di purificazione dell'enzima: esso si presta all'isolamento di tutte le proteine funzionalmente attive indipendentemente dall'origine e dalle caratteristiche chimico-fisiche. Questo è convalidato anche dall'identità dello spettro delle forme elettroforeticamente multiple dell'enzima negli estratti grezzi e nelle preparazioni altamente purificate così ottenute.

Inoltre la possibilità di estrarre selettivamente ed in modo quantitativo tutte le proteine funzionalmente identiche presenti anche in modesta concentrazione negli estratti di tessuto o nei liquidi biologici rende il metodo estremamente interessante per l'indagine biochimica clinica.

Risultati del progetto n. 4

Capo del progetto e collaboratori scientifici:

Prof. Carlo Giuntini, Dr. Ferruccio Fazio, Dr. Anna Maria Santolicandro,

Titolo del progetto: Sviluppo dell'impiego dei radioisotopi a vita breve nella diagnostica morfo-funzionale quantitativa polmonare nell'uomo.

Messa a punto di metodi scintigrafici con rivelatore mobile e fisso ed impiego di microsferi di albumina marcate per lo studio della distribuzione del volume di sangue polmonare.

Negli ultimi anni si è avuta la tendenza a sostituire nella tecnica scintigrafica i rivelatori a testa mobile focalizzante con i rivelatori a testa fissa (Gamma-camera e simili). E', quindi, opportuno procedere ad una valutazione della distribuzione di efficienza di questi ultimi in relazione alle esigenze della scintigrafia polmonare.

In primo luogo, con le dimensioni attualmente disponibili delle teste rivelatrici, per la scintigrafia polmonare è necessario ricorrere ai collimatori divergenti al fine di inquadrare ambedue i polmoni nel campo visivo della Gamma-camera. Nella figura 1, che si riferisce all'americio-241, che ha un'energia simile allo xenon-133, si osserva che: 1) le zone polmonari vicine al rivelatore sono viste con una efficienza molto superiore rispetto alle lontane; 2) il rivelatore addiziona, per così dire, le radiazioni provenienti dalle varie zone polmonari poste sull'asse di rivelazione; 3) la risoluzione in senso laterale è buona; 4) il contributo del polmone controlaterale è piccolo. Si tratta, quindi, di una rivelazione radioattiva con buona selettività rispetto ai campi laterali, ma con scarsa selettività nel senso della profondità di campo visivo.

Passando al cobalto-57, figura 2, che ha un'energia simile al tecnezio-99m, si possono fare le stesse osservazioni che per l'americio-241, tranne che per il contributo del polmone controlaterale che è maggiore.

Infine, figura 3, nel caso dello iodio-131, che ha un'energia simile all'iodio-113m, non solo il contributo del polmone controlaterale diventa importante, ma anche la selettività rispetto ai campi laterali è meno buona. Va tuttavia segnalato che, mentre coi livelli energetici dello iodio-131, non si osservano artefatti negli scintigrammi polmonari, coi livelli energetici del tecnezio è frequente osservare irregolarità di distribuzione dovute alle strutture delle pareti toraciche e dei cingoli scapolari.

Si può quindi concludere che, mentre coi livelli energetici più alti la selettività della Gamma-camera è modesta, sia nel senso della profon-

dità che in quello laterale, tuttavia, se si richiede una misura della radioattività contenuta nelle varie parti del polmone, è necessario ricorrere a tali livelli energetici. Più in generale, si deduce da questi dati che la scelta del livello energetico da impiegare in un determinato studio può essere diversa a seconda dei fini dello studio stesso ed è opportuno ponderare accuratamente ciò che si vuole misurare con la distribuzione di efficienza della Gamma-camera per i vari isotopi affinché la scelta dell'isotopo sia la più razionale possibile.

L'elemento più significativo è il livello energetico dell'isotopo impiegato e non il tipo di collimatore usato. Nella figura 4, relativa al cobalto-57 ed ad un collimatore con 4000 fori, si osserva che la distribuzione delle linee di isoefficienza è in tutto simile a quella ottenuta per lo stesso cobalto-57 col collimatore divergente.

Questi studi sulla distribuzione dell'efficienza della Gamma-camera sono stati eseguiti per un'area quadrata del cristallo con un lato di 1 cm. Nella figura 5, la risoluzione della Gamma-camera, espressa come ampiezza della curva di efficienza corrispondente a metà dell'efficienza massima per un dato piano, sull'ascissa, è confrontata con l'area del cristallo presa in esame, sulle ordinate.

Si conclude osservando che non c'è molto da guadagnare in termini di risoluzione effettiva, scendendo ad analizzare aree del cristallo più piccole di 1 cm quadrato.

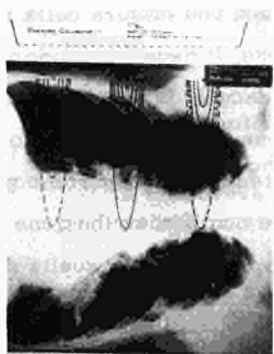


Fig. 1



Fig. 2



Fig. 3



Fig. 4

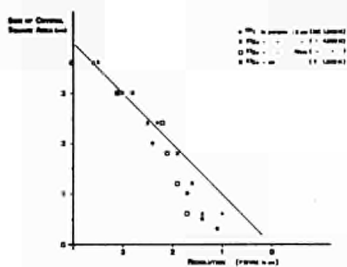


Fig. 5

Risultati del progetto n. 5

Capo del progetto e collaboratori scientifici:

Prof. Attilio Maseri, Dr. Carlo Contini, Dr. Antonio Pesola, Dr. Antonio L'Abbate, Dr. Paolo Mancini, Dr. Mario Marzilli, Dr. Anna Maria Ballestra, Dr. Rita Mimmo, Dr. Anna Maria Paci.

Titolo del progetto: Sviluppo dell'impiego di radioisotopi a vita breve nella diagnostica morfofunzionale quantitativa del miocardio.

1. Alterazioni della perfusione miocardica regionale nell'angina pectoris.

Lo studio della perfusione miocardica regionale è stato condotto mediante l'impiego di una gamma camera (Pho Gamma III Nuclear Chicago) collegato ad un calcolatore (HP 2116B). Questo sistema permette di analizzare la distribuzione iniziale nel miocardio di un tracciante diffusibile gamma emittente iniettato selettivamente in una arteria coronaria ed il suo washout dalle varie zone dello scintigramma.

Come tracciante è stato usato lo $^{133}\text{Xenon}$ che presenta convenienti caratteristiche isotopiche ma che avendo un coefficiente di partizione miocardio-tessuto adiposo di 10-1 e quindi una significativa diffusione nel grasso sopraepicardico non permette una quantizzazione del flusso in ml/min per unità di tessuto.

D'altra parte, finchè non sarà disponibile un tracciante più adatto (prodotto da un ciclotrone), è possibile ottenere informazioni semiquantitative sul comportamento del flusso regionale in diverse condizioni emodinamiche nello stesso paziente dalle variazioni della distribuzione iniziale e della velocità di washout regionali ottenute con iniezioni successive del tracciante.

Pertanto abbiamo studiato le alterazioni della perfusione regionale durante angina pectoris in 10 soggetti con ostruzione critica della coronaria discendente anteriore con lesione unica o predominante in condizioni basali, durante angina indotta da pacing e dopo la scomparsa dell'angina per l'interruzione del pacing e/o la somministrazione di nitroglicerina. In 5 pazienti l'angina è stata indotta due volte: la seconda volta aumentando bruscamente la frequenza cardiaca immediatamente dopo l'iniezione intracoronarica del tracciante.

Risultati

In 8 su 10 pazienti l'iniezione del tracciante dopo l'induzione dell'angina mediante pacing ha mostrato una marcata riduzione della distribuzione iniziale del tracciante nella zona distale alla ostruzione coronarica rispetto al controllo indicando una locale riduzione della perfusione rispet

to alle zone circostanti.

Quando l'angina è stata indotta mediante un brusco aumento della frequenza dopo l'iniezione intracoronarica (cosicché il miocardio era già uniformemente "marcato") il washout è risultato marcatamente rallentato nelle zone che avevano mostrato un deficit nella distribuzione iniziale quando il tracciante era stato iniettato dopo l'induzione dell'angina.

Questi studi forniscono una serie di dati originali sulla natura regionale delle alterazioni della perfusione miocardica nell'angina pectoris. Inoltre essi indicano che nella zona ischemica la perfusione durante angina non solo non è incapace di aumentare fino ad adeguarsi alle richieste metaboliche ma si riduce rispetto ai valori precedenti suggerendo l'intervento di fattori funzionali (meccanici, umorali o nervosi) nell'insufficienza coronarica acuta.

2. Scintigrafia miocardica selettiva per la valutazione dell'estensione dell'area infartuata.

L'iniezione intracoronarica di piccole quantità di microsfele di albumina umana marcata con ^{99m}Tc o con ^{113m}In per la scintigrafia miocardica e risulta immune per il paziente.

L'impiego di questa tecnica in associazione con le tecniche emodinamiche di cateterismo cardiaco per la coronarografia selettiva e la ventricolografia è in grado di fornire informazioni sulla localizzazione ed estensione delle zone di fibrosi miocardica postinfartuale.

La scintigrafia è stata eseguita mediante:

a) Gamma camera (Pho Gamma III Nucleare Chicago); b) Rectilinear scanner; c) Tomo camera (Nuclear Chicago).

Sono stati studiati 22 pazienti, 4 con coronarografia normale, 6 con lesioni coronariche ma senza infarto e 12 con pregresso infarto miocardico.

Risultati

La distribuzione del tracciante appare uniforme in assenza di un pregresso infarto. E' possibile evidenziare il miocardio atriale con il setto interatriale, il setto interventricolare, le due cavità e la parete anteriore e posteriore dei ventricoli.

L'iniezione di microsfele marcate con traccianti diversi permette di valutare la distribuzione miocardica della coronaria dx. e sin. e la presenza di circolo collaterale intercoronarico. La sede e l'estensione delle aree fibrotiche postinfartuali vengono rivelate con soddisfacente chiarezza, ed è possibile così ottenere utili indicazioni chirurgiche.

Il confronto tra i tre sistemi di rilevazione dello scintigramma miocardico indica che la Tomo camera non presenta particolari vantaggi rispetto alla Gamma camera ed allo Scanner.

Questi due sistemi, sui dati finori raccolti, sembrano equivalersi.

Risultati del progetto n. 6

Capo del progetto e collaboratori scientifici:

Prof. Claudio Bianchi, Dr. Antonio Coli, Dr. Roberto Palla, Dr. Mario Bonadio

Titolo del progetto: Sviluppo dell'impiego dei radioisotopi a vita breve nella diagnostica morfofunzionale quantitativa del rene e dell'ipertensione arteriosa.

Messa a punto di metodi di screening del danno renale monolaterale mediante tecniche scintigrafiche quantitative (C. Bianchi, R. Palla, M. Bonadio).

Al fine di superare le difficoltà ed i rischi delle tecniche disponibili per lo screening del danno renale monolaterale è stato progettato un semplice apparato che consente di ottenere la scintigrafia renale quantitativa.

Descrizione tecnica

Tale apparato, che completa un usuale scanner del commercio, permette di effettuare, simultaneamente alla registrazione scintigrafica, due conteggi separati della radioattività in due campi di esplorazione rispettivamente a destra e a sinistra della linea spondiloidea del paziente. Esso consiste in un microinterruttore che è tenuto in posizione aperta e chiusa da una camma rettilinea, parallela alla linea di scorrimento del rivelatore e posizionabile a piacere lungo la linea stessa. Un circuito di collegamento del microinterruttore a due scale di conteggio fa corrispondere alle due diverse posizioni del microinterruttore l'effettuazione del conteggio sull'uno o l'altro degli scalers. Nella figura 1 sono schematizzati lo scanner, che comprende l'apparato descritto costituito da camma e microinterruttore, i due scalers e la proiezione del rivelatore sul rene. In alto al rivelatore è situato sopra il rene sinistro. In questa posizione il microinterruttore determina la connessione tra rivelatore e scaler sinistro, mentre il destro è escluso. Successivamente spostando il rivelatore trasversalmente al paziente, la camma determina lo scatto del microinterruttore; viene in tal modo escluso lo scaler sinistro ed entra in connessione quello destro.

Casistica e risultati

L'apparato descritto è stato verificato in 10 soggetti normali ed in 2 pazienti ipertesi in cui i risultati ottenuti con la scintigrafia quantitativa sono stati confrontati con il filtrato glomerulare (FG), e la portata renale

plasmatica effettiva (PRPE) di ciascun rene, misurati mediante cateterismo ureterale bilaterale. Nei soggetti normali il rapporto di fissazione del tracciante (^{197}Hg -neohydrin) tra rene sinistro e rene destro è risultato di 0.97 ± 0.10 . Nei soggetti ipertesi tale rapporto di fissazione è apparso simile a quello del FG e della PRPE tra i due reni (rispettivamente 0.48, 0.52, 0.61 nel primo paziente e 1.02, 0.92, 0.99 del secondo).

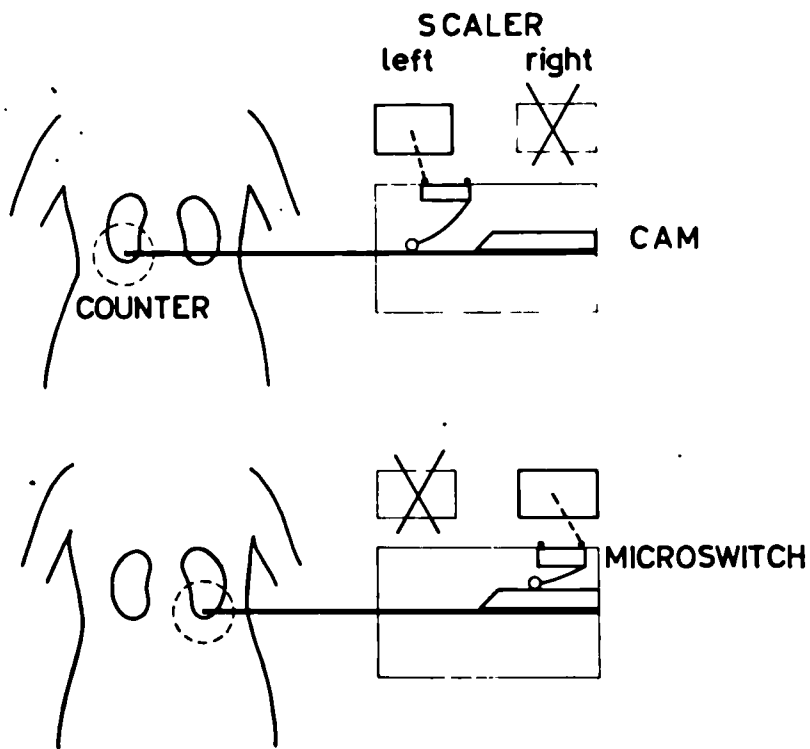
Questi dati depongono per la validità del metodo descritto per lo screening del danno renale monolaterale.

La misura del FG mediante ^{140}La -DTPA (C. Bianchi, A. Coli, R. Palla, con la collaborazione di A. Lo Moro)

Per valutare il meccanismo di eliminazione renale del ^{140}La -DTPA la sua clearance è stata misurata simultaneamente a quello del ^{131}I -hypaque, della creatinina endogena e dell'urea in 13 soggetti con diversa funzione renale eseguendo le misure in condizioni di diuresi basale (flusso urinario medio 1.39 ± 0.80 ml/min) ed in diuresi osmotica da mannitolo (12.50 ± 2.54 ml/min). I risultati sotto riportati dimostrano che il ^{140}La -DTPA è escreto esclusivamente mediante filtrazione glomerulare e può quindi essere usato per la misura del FG nell'uomo.

Clearances medie e rapporti delle clearances a basso ed alto flusso urinario

<u>flusso urinario</u>	<u>^{131}I-hypaque</u>	<u>^{140}La-DTPA</u>	<u>Creatinina</u>	<u>Urea</u>
basso	100.56 \pm 19.89	102.46 \pm 20.12	115.89 \pm 25.99	45.39 \pm 19.96
alto	97.61 \pm 16.04	96.64 \pm 17.72	115.08 \pm 25.73	67.81 \pm 17.12
<u>cl. basso flusso</u>	1.03 \pm 0.09	1.06 \pm 0.10	1.00 \pm 0.09	0.67 \pm 0.18
<u>cl. alto flusso</u>				



Didascalia della figura 1

Apparato per la scintigrafia renale quantitativa: schema del suo funzionamento.

Vertragspartner der Kommission:

Gesellschaft für Strahlen- und Umweltforschung
Institut für Hämatologie

Nr. des Vertrags: O89-72-1 BIAD

Leiter der Forschungsgruppe:

Priv.-Doz. Dr. Stefan Thierfelder

Allgemeines Thema des Vertrags:

Nuklearmedizinische Hämatologie (Proj. 7-12).

(Proj. 1-5 über Strahlenbiologische Hämatologie und Immunologie sind unter Kapitel III "Forschungstätigkeit Strahlenschutz" aufgeführt).

The immunological department of the institute concentrated on questions concerning the treatment of the consequences of radiation exposure. It isolated a fraction active against T-cells from heterologous antibrain serum, which suppressed acute secondary disease (Proj.No.5) and produced T-cell deprived mice simulating the nude strain (Proj.No.3). Furthermore it documented the low degree of chimaerism in mice conditioned with cyclophosphamide (Proj.No.2) and developed a non-lethal conditioning treatment of partial body irradiation and ALS (Proj.No.4).

In radiation hematology the long term studies on the morphological consequences of a treatment with radiophosphorus were continued (Proj.No.1). The basic studies of a new in vitro method for determining DNA synthesis rates of individual, morphologically classifiable human cells have been completed and applied to various forms of human anemia (Proj.No.10).

Ergebnisse des Projekts Nr. .7.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Dr.G.Brehm, Dr.H.Pfisterer, Dipl-Phys.W.Ruppelt

Titel des Projekts :
Kinetics and Clinical effects of blood cells isolated by
NCI-IBM Cell Separator and stored by cryobiological methods

In 1972 our main intention was to substitute granulocytopenic patients suffering from life threatening infects with granulocytes. To procure a sufficient amount of granulocytes for transfusion with the NCI-IBM blood cell separator we tried to increase the yield obtained by means of former methods. The use of an erythrocyte sedimentation accelerator and a special dosage of the anticoagulant enhanced the yield of a six hours' run to an average of 1.6×10^{10} granulocytes. The application of prednisolon to the donor further increased the mean yield to 5.5×10^{10} granulocytes in the same time (1). A method for careful preparation of the withdrawn cell concentrate was developed which allows to eliminate the erythrocytes almost completely (granulocytes: erythrocytes = 1 : 2) and to retransfuse them to the donor. Thus it is possible to substitute a patient for a long time with granulocytes from one single donor.

The viability of the isolated cells, proved by evaluating the half life of $DF^{32}p$ labelled granulocytes, has shown to be equivalent to the viability of non-isolated, in whole blood $DF^{32}p$ labelled granulocytes.

In a long-term study about the clinical effect of granulocyte transfusions we have substituted 5 patients over a period of up to 18 days so far. In two cases the bone marrow activity recovered, the dangerous stage of aplasia was overcome.

Ergebnisse des Projekts Nr. .8.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Prof.Dr.W.Stich, Dr.D.Schmidt, Dr.M.Schmidt

Titel des Projekts :
Regulation and disturbances of hemoglobin metabolism in man

In 1972 a study was concluded of the fetal synthesis of heme in different periods of erythropoiesis from the mesenchymale to the myeloic. We found an accelerated synthesis of heme-precursors in red cells during the whole fetal period, mostly in the stage of mesenchymale erythropoiesis, which is related to an accelerated maturation of extramyeloic erythropoiesis as it was shown in animals. Corresponding to the fetal age the porphyrin concentration of the liver increases in relation to liver erythropoiesis, rising synthesis of heme in the hepatocytes and storage and elimination of porphyrins by the liver. The synthesis of heme in the spleen is not important at any stage of erythropoiesis.

Further studies concern the inhibition of synthesis of heme by lead. The investigation of the urinary porphyrins of low-contaminated and severe lead-intoxicated human adults demonstrated a characteristic change in the relation and quantity of heme precursors with significant correlation to the stage of intoxication. This gives evidence to the effect that lead inhibits, especially pentacarboxylic porphyrins, apart from the known enzymatic blockades and also selectively inhibits decarboxylation of porphyrin-intermediates. The copro/uroporphyrin ratio may also be of diagnostic value with a normal average of 3.3, increasing to an average of 30.5 at lead intoxication. A modified method to separate porphyrin intermediates in quantities of less than 0.005 µg by bi-dimensional thin layer chromatography was helpful in these analyses.

The investigation of 21 policemen, intensively exposed to traffic in Munich, revealed moderately increased levels of blood lead and mild signs of a disturbance of biochemical pathways caused by lead. Blood lead was enhanced by 34 %, erythrocyte protoporphyrin by 25 % and urinary Ala by 22 %, differing significantly from the values of inhabitants of Munich not especially exposed to lead. In chromosome analyses of these policemen no chromosomal damage was detected.

Ergebnisse des Projekts Nr. .9.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Dr.H.-J. Zeitler, E.Stadler

Titel des Projekts :
Synthesis of labelled heme precursors and isolation of
heme synthesizing enzymes

To enable the elucidation of the heme- and bilirubin biosynthesis, of the enteral resorption of porphyrines and of the enzymopathologies in the biosynthesis of hemoglobins in the hemopoietic system in man some radioactive labelled heme precursors and products of hemoglobin breakdown have to be synthesized and enzymes involved in these reactions have to be isolated and analyzed for their amino acid sequence.

The purpose of this project is the isolation and purification of δ -ALA-synthase and δ -ALA-dehydratase (porphobilinogen synthase) from human tissues (bone marrow, reticulocytes, liver) and the elucidation of their structure (amino acid sequence, analysis of metal content, 3-dimensional X-ray analysis of the crystallized enzymes), so that we can show by means of the fingerprinting technique the enzyme defects as molecular biological cause of hemoglobinopathies and porphyrias. To analyze the above mentioned enzymes, their content in various tissues and the regulation of porphyrin- and bilirubin metabolism with C-14-labelled heme precursors (δ -ALA, porphobilinogen), we have made a program for the preparative isolation and purification (gel filtration, ammonium sulfate fractionation, gel isoelectric focusing) and crystallization of the two enzymes and their subunits from rabbit liver (with and without enzyme induction with 2-allyl-2-isopropylacetamid), and from a special strain of the photosynthetically grown microorganism *Rhodospseudomonas sphaeroides*.

It was possible to eliminate the considerable difficulties in selecting and culturing suited strains of the photosynthetically grown microorganism *Rh.sph.* after various experiments.

Ergebnisse des Projekts Nr. 10.

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Dr.P.Dörmer, Dr.P.DahrJr., Dr.W.Brinkmann

Titel des Projekts :
Erythropoiesis and kinetics of erythroblasts in anemias
and aplasias of man.

The basic studies of a new in vitro method for determining DNA synthesis rates of individual, morphologically classifiable human cells have been completed. This method consists of 3 new techniques:

1) A procedure of in vitro incubation of suspended bone marrow cells. Thymidylate synthetase is blocked by FUDR. DNA synthesis, however, proceeds undisturbed, DNA-thymine being recruited from exogenous ^{14}C -thymidine only.

2) Quantitative autoradiography of single cells. The autoradiographic technique has been standardized utilizing ^{14}C -standard sources on the autoradiographs. Thereby, grain counts can be converted into incorporated amounts per cell of ^{14}C -thymidine. This corresponds to DNA synthesis rates if the incubation procedure with FUDR is applied, and from the latter the DNA synthesis time of individual cells can be calculated (see Fig.1). The results in the field of quantitative autoradiography will be the basis for the manual and a lecture to be held at the 3rd EMBO course on Micromethods in Molecular Biology, Göttingen, September 1973.

3) A grain counting design by incident light microphotometry (3). Data obtained by photometry are punched on a tape and processed by a computer. Frequency distributions and statistical analyses are performed in this way.

In a study of normal human and rat erythroblasts using this method it was found that DNA synthesis time is lengthened with increasing maturity of the cells. Since the ^3H -thymidine labeling index decreases correspondingly, a lengthening of erythroblast generation times with maturation can be stated. In the course of maturation thymidine kinase activity also decreases significantly.

Various forms of human anemia have been investigated, the results being currently published. Fig.1 represents the DNA synthesis time of erythroblasts in a case of blastic crisis of chronic myelocytic leukemia. These results were presented at the 2nd International Symposium on Metabolism and Membrane Permeability of Erythrocytes, Thrombocytes and Leukocytes, Vienna 1972. The calculations of proliferation kinetics derived from these results suggest that the anemia in this case of leukemia is not the result of a decreased proliferative activity of the erythroblasts. The disturbance of erythropoiesis should rather be expected at the stage of the erythropoietic stem cell.

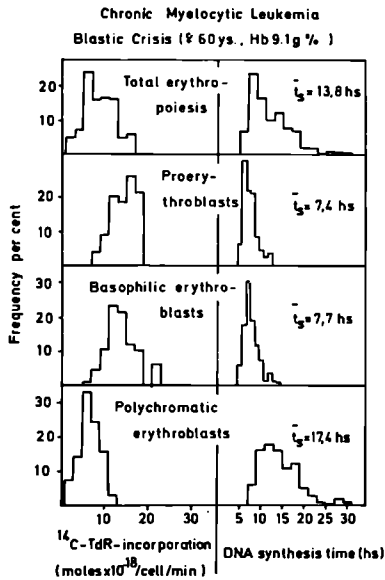


Fig. 1: DNA synthesis rates (left side) and duration of DNA synthesis (right side) in 3 morphologically determined erythroblast compartments and in total erythropoiesis. The distributions of DNA synthesis rates are comparable to symmetric distributions, in favorable cases even to normal distributions. This speaks in favor of a constant DNA synthesis rate in the different compartments. Consequently, the distributions of DNA synthesis time are skewed to the right resembling log-normal or reciprocal-normal histograms.

Ergebnisse des Projekts Nr. .11

Leiter des Projekts und wissenschaftliche Mitarbeiter :

Prof.I.Boll

Titel des Projekts :

Haemopoiesis in bone marrow cell culture

One of the main results of our work was that the quantitative counting of the in vitro method corresponded fully to the kinetics in vivo obtained by autoradiographic methods. After freezing and defreezing, the proliferation of the erythropoiesis continued for several days in our human bone marrow cultures. A certain synchronization could be obtained by cooling or with inactive thymidine. It was thus theoretically proved that our experiments were transferable to the human organism.

The investigations about the influence of cytostatica and the erythropoietin on human bone marrow clot cultures, initiated already a few years ago, could be nearly completed. Furthermore studies comparing the influence of cytostatica on normal and leukemic bone marrow in vitro have been started. The investigations about a serum-fraction of patients with Banti-syndrome, about vitamins and about two corticosteroids on human bone marrow in vitro were terminated, but other serum-fractions of patients with Banti-syndrome and other corticosteroids are still being studied. To continue this work is of major interest, as promising results are in view, and all our former work has to be seen under this general aspect.

The evaluation of our oil immersion phase contrast filming has been the main aim of our research in 1972:

Our examinations about leukemic blasts, erythroblasts and erythrophagocytosis were terminated so far by compiling the results on the maturation of leukemic myeloblasts and the system of monocytes and macrophages in humans. It appears to us as a logical consequence to gather the phase contrast films about the kinetics of basophilics and mast cells, of the eosinophilics and of the stem cells, and to continue film them. There exist several observations regarding the interchangeability of the stem cell pool: myeloblasts, monoblasts, and proerythroblasts. Everything should be done to continue to work on the kinematographic documentation of the "curriculum vitae" of the single cells, i.e. their kinetic behaviour and their transformation from one maturation stage to another.

Ergebnisse des Projekts Nr. 12

Leiter des Projekts und wissenschaftliche Mitarbeiter :
Prof.Dr.H.Ehrhart und Dr.Wolf-Hornung

Titel des Projekts :
Metabolism of labelled tumor inhibiting substances
in leukaemia and cancer.

Leukocytes in human chronic myelogenous and lymphatic leukemia contain more NAD and less lipid constituents than normal leukocytes. An early effect of treatment of chronic myelogenous leukemia with single courses of an ethylenimine agent (C 73) was the change of these constituents toward normal, associated with subnormal concentration of ATP. These levels persisted during complete or partial remissions. Busulfan therapy produced similar but smaller effects. Such effects were not produced in chronic lymphatic leukemia by treatment with cyclophosphamide. Experiments with the transplanted Shay myeloma in the rat showed that therapeutically effective doses of C 73 also drastically reduced the NAD and ATP concentrations in these tumors.

The response of Ehrlich-Ascites-Tumor-cells in mice to treatment with Vincristine in two different dosages was tested by cellkinetic investigations. The number of mitotic cells increased under Vincristine treatment and this increase was more pronounced with higher dosage. Utilising Impule-Cytophotometry, cells with double DNA content appeared more frequently and at the cost of those with single DNA content under higher Vincristine dosage. This shift of DNA contents lasted at least 55 hours after single Vincristine application. This finding suggests that higher doses of Vincristine cause an increase of polyploid EAT-cells.

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I. Boll, R. v. Wächter und J. Meyer-Burg

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Klin. Wschr. 50, 510 (1972)

I. Boll

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(Lehmann Verlag, München 1972)

I. Boll und V. Correll

Morphologische Studie zum Verhalten von Knochenmarkzellen in vitro. VI. Verlaufsbeobachtungen an Erythroblasten.
Blut XXIV, 102 (1972)

W. Brinkmann und P. Dörmer

In vitro-Verfahren zur Bestimmung der DNS-Synthese-Dauer einzelner Zellen. Biochemische Voraussetzungen und Ergebnisse.
Histochem. 30, 335-343 (1972)

R. Burkhardt

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Blut 25, 185-189 (1972)

P. Dörmer und W. Brinkmann

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D. Huhn und F. Schmalzl

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D. Huhn und F. Schmalzl

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Groß/van de Loo, Springer-Verlag (1972)

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Atypical (monomyelocytic) myelogenous leukemia. Cytochemical, electron microscopic and biochemical investigations. Acta haemat. 48, 72 (1972)

D Schmidt, W. Stich und G. Dech

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und W. Stich

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MMW 114, 1761-1763 (1972)

D. Schmidt und W. Stich

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D. Schmidt, G. Dech, R. Fikentscher und W. Stich

Über die fetale Hämsynthese in verschiedenen Blutbil-
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Blut 25, 85-96 (1972)

H. Schraub, H. Pfisterer, W. Ruppelt, W. Gross und
H. Bolland

Verhalten DF³² P-markierter Granulozyten nach homologer
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Klin. Wschr. 50, 570-573 (1972)

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NEUTRONENDOSIMETRIE

NEUTRON DOSIMETRY

DOSIMETRIE DES NEUTRONS

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

Nr. des Vertrages: 113-72-1 BIOC

Leiter der Forschungsgruppe: Dr. Georg Burger

Allgemeines Thema des Vertrages: Die Berechnung und Messung
von Energie-Dosis und Neutronenspektren innerhalb
eines biologischen Objektes im Hinblick auf die The-
rapie mit schnellen Neutronen

The program is concerned with the determination of the biologically effective neutron dose inside human phantoms. It includes neutron and gamma-deep penetration calculations and measurements with various detectors.

Calculations have been performed for cylindrical homogenous phantoms by means of the multi-group code DOT 2.

As in therapy usually narrow beams are applied, also collimation of neutron was investigated. The effect of the collimator was characterized by the different isodosedistributions for an ideal narrow beam and a real beam, derived by applying the transport calculations to the total system target-collimator-phantom.

In the past only the neutrons could be taken into consideration. Inside the phantom the local neutron spectra and the kerma have been calculated. The data library for the production and transport of the gamma component was established and the referring parts of the code have been tested.

From the local neutron spectra several correction factors for the application of gaseous chambers for the measurement of dose inside phantoms have been derived. The experimental setup for the ICRU-intercomparison-measurements was provided and the calibration of the instruments prepared.

The efforts in spectrometry of fast neutrons are continued.

Ergebnisse des Projektes Nr. 1.

Leiter des Projektes und wissenschaftliche Mitarbeiter:

G. Burger, F. Grünauer, E. Maier

Titel des Projektes: Calculation and measurements of the spectral neutron and gamma quantum flux density and kerma in free space and within phantoms

As a first step only the neutron component was calculated for irradiation of homogenous cylindrical shaped phantoms by 15 MeV-neutrons using the multigroup transport code DØT 2.

For all neutron transport calculations concerning the phantom, the composition of standard man tissue according to ICRU 19 was used. All other components were neglected. From the calculations the neutron spectra for each spatial mesh point of the assembly have been obtained. From this the kerma was calculated by multiplying the spectra with the energy dependent fluence-to-kerma factor for standard man and integration over the total energy range. Except for the buildup-regions near boundaries the kerma equals the dose.

In order to study the influence of insufficient collimation and collimator leakage on the dose distribution in the phantom, one must know the dose distribution that would result from an ideal, geometrically limited narrow beam without leakage neutrons. Fig. 1 shows results for the axial depth dose for several diameters d_a of an ideal aperture and for free space irradiation of the phantom. In order to compare the results with the Oak Ridge data, the latter, as given in NCRP 38 for parallel beam, were multiplied with $(\frac{SSD}{SSD+t})^2$ and also plotted. Of course this is only an approximation. In addition the phantoms in NCRP 38 and the one used here have quite different shapes and also slightly different compositions.

Yet the differences are not too high and can be explained. Along the axis the values for the real collimator correspond with those for an ideal aperture as can be seen from fig. 2. It shows the isodose distributions for both an ideal and real collimation. The isodoses are normalized at 10 cm depth. Down

to the 40% isodose there is no difference surmounting the errors of calculation. Below this the scattering of neutrons out from the collimator duct and its effect especially at low phantom depth can clearly be seen.

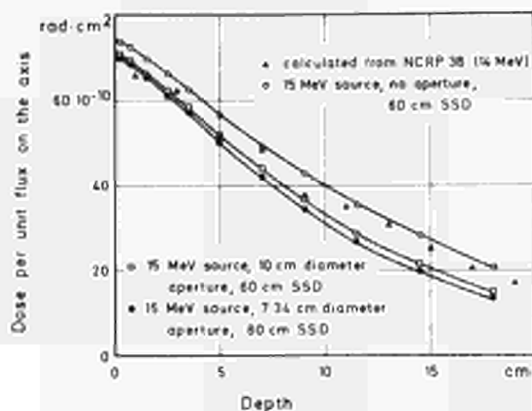


Fig. 1:
Axial depth dose curves for various apertures and inter-comparison with NCRP 38 values.

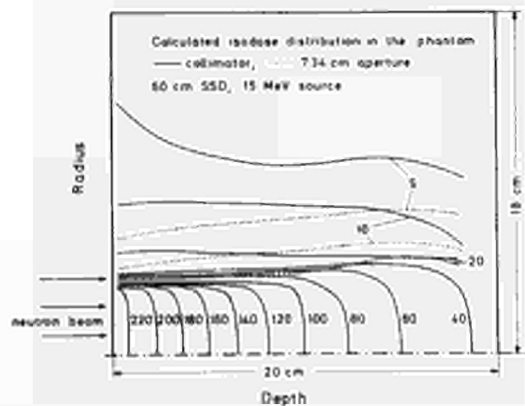


Fig. 2:
Isodosechart for the phantom, real collimator and test calculation with an aperture, 15 MeV source.

Literature:

Grünauer, F: Calculation of a neutron beam collimator for radiotherapy
Proc. First Symp. on Neutron Dos. Biol. Med.
EUR 4896 d-f-e
Luxembourg 1972

Vertragspartner der Kommission:
Gesellschaft für Strahlen- und Umweltforschung
München
Nr. des Vertrages: 113-72-1 BIOC
Leiter der Forschungsgruppe:
Prof. Dr. W. Pohlitz
Allgemeines Thema des Vertrages:
Calculation and Measurement of Absorbed Dose
and Neutron Spectra inside a Biological Object
with Reference to Fast Neutron Radiotherapy

For the determination of back scattering factors of neutron doses with ionization chambers the irradiation arrangement and measuring devices were prepared. For the production of neutrons (D-T reaction) a Brown Boveri 600 kV accelerator is employed. The beam characteristics, especially the accompanying gamma radiation produced by the neutron in the target assembly was investigated.

Ergebnisse des Projektes Nr. 1

Leiter des Projekts und wissenschaftliche

Mitarbeiter: Dr.H.Kühn, Dipl.-Phys.A.Müller

Titel des Projekts: Determination of back scatter factors on interfaces

Several ionization chambers have been developed for the determination of the absorbed dose within and in front of phantoms irradiated with fast neutrons. The ionization chambers differ in their sizes and in their wall materials. The sensitive volumes of the ionization chambers range from about 50 cm^3 to 2.6 cm^3 . The wall materials were tissue equivalent material, polyethylene, graphite, aluminium, and magnesium. The radiation sensitivities of the instruments can either be calculated from the sensitive volume or determined experimentally with gamma radiation of a well known dose given at the interesting place. We preferred a calibration of the homogeneous chambers within a graphite phantom irradiated with Co-60 gamma radiation. From this calibration the gamma and neutron sensitivities have been calculated for the interesting materials. (KÜHN 1972). Further comparison measurements with a 14 MeV neutron beam have been performed especially with non homogeneous instruments. The reproducibility $\Delta Q/Q$ of charge measurements with ionization chambers is given by $\Delta Q/Q = 0.002$. The uncertainty in neutron dose determination is about $\pm 10\%$. A special gas supply for the ionization chambers was built. For the measurements of dose back scattering factors a tissue equivalent ionization chamber and a fission chamber (U-238) were employed as monitors. The charges of both monitors produced by a mixed neutron and gamma field are notified on two separate digital displays. Since the fission chamber is gamma insensitive a variation of the gamma dose during the irradiation indicates a change of the ratio of the digital displays.

The ratio of the gamma dose and neutron dose $D_{TE\gamma}/D_{TE^n}$ was measured in free air with paired ionization chambers for different deuteron currents at the tritium target of the Brown Boveri accelerator. For deuteron currents within the range of 0.5 mA and 1.6 mA it followed $D_{TE\gamma}/D_{TE^n} < 0.05$, this ratio is constant within 2%. The relation between target distance and dose obeys the squared distance law for target distances between 10 cm and 200 cm. This relation shows that the dose contribution of scattered radiation by the walls of the room may be neglected at the local arrangement chosen. At this stage of investigations dose back scattering factors have not been determined since fluence and depth dose measurements in homogeneous phantoms have been studied firstly (KUHN et al. 1972, MÜLLER 1972).

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- Müller, A., "Bestimmung der Tiefendosis in einem gewebeäquivalenten Phantom bei Bestrahlung mit schnellen Neutronen", Diplomarbeit an der Johann-Wolfgang-Goethe-Universität, Frankfurt am Main 1972.

Contractor: MEDICAL RESEARCH COUNCIL
Contract No. 113-72-1 BIOC
Head of research team: D.D.VONBERG
General subject of Contract: CORRELATION OF PHYSICAL AND
BIOLOGICALLY EFFECTIVE DOSE OF FAST NEUTRONS

Mammalian cells have been exposed to the fast neutron beam from the MRC cyclotron at various depths in a phantom and with the phantom removed. The cells were in suspension and were bubbled with air or nitrogen during irradiation. The results have been correlated with measurements of absorbed dose at the depths used.

Other cells have been exposed to the beam with and without secondary charged particle equilibrium, again in the presence and absence of oxygen. This will enable us to obtain information on the separate radiobiological properties of the recoil proton and heavy recoil components of the dose.

Results of Project No. 1.

Head of Project and scientific staff: D.K.Bewley,
R.J.Berry.

Title of Project: Measurement of biologically effective
dose of fast neutrons.

Mouse lymphoma cells were irradiated in suspension, as described by McNally and Bewley (Brit.J.Radiol. 42, 289, 1969). The field size was 16 x 16 cm. (except as noted below) and the surface of the phantom was at 120 cm. from the target. The results are as follows:- (numbers in brackets are 95% confidence limits):-

Depth in phantom cm.	Extra-polation No.	D ₀ in		
		Air	Nitrogen	OER
No phantom	1.8	78 (65-99)	149 (138-234)	1.9 (1.6-2.3)
1.5	5.5	68 (60-80)	112 (96-133)	1.6 (1.4-1.8)
8.7*	1.1	94 (79-115)	134 (113-163)	1.4 (1.2-1.7)
15.5	2.3	74 (67-82)	107 (98-119)	1.4 (1.3-1.6)
23.0	1.2	80 (33-182)	-	-

*Using 9.5 x 9.5 cm. field (earlier result).

It will be seen that the OER in the phantom is less than in air, as Berry has found previously. This is the first experiment in which Berry has used the geometry of McNally and Bewley instead of the less satisfactory arrangement which he had used previously. Confirmation of the result with a new and improved geometry confirms that it is unlikely to be an artifact. The change in OER is due to an increased sensitivity under hypoxic conditions in the phantom.

Results of Project No. 2.

Head of Project and scientific staff: D.K.Bewley,
N.J.McNally.

Title of Project: Effect of secondary charged-particle
spectrum on biological response.

Description of results

Cultured rat fibrosarcoma cells were exposed to the neutron beam in the presence and absence of secondary charged-particle equilibrium. The cells were plated on melinex and placed facing the beam; sheets of aluminium or tissue-equivalent plastic were put over them. The oxygen enhancement ratio was found to be 1.4 in the presence of charged-particle equilibrium and 1.0 in its absence. The OER of the recoil proton component alone is about 2.2.

Calculations of absorbed dose under the two conditions are now being made in order to assess the RBE values.

Contract No. 113-72-1 BIOC

Radiobiological Institute TNO, Rijswijk (ZH), The Netherlands

J.J. Broerse

Calculation and measurement of absorbed dose and neutron spectra inside a biological object with reference to fast neutron radiotherapy

In order to obtain quantitative information on the effectiveness of fast neutrons for clinical applications, responses of pulmonary metastases in patients are being studied at the Radiobiological Institute after irradiation with 15 MeV neutrons or gamma-rays. The dosimetry studies for this project are primarily concerned with depth-dose measurements, using different phantoms of the upper part of the human body, in order to obtain knowledge of the beam profile and iso-dose curves within patients.

The total absorbed dose at various sites was measured using tissue-equivalent ionization chambers, whilst an estimate of the gamma contamination was derived using an additional chamber which was relatively insensitive to neutrons. Dosimetry data obtained from measurements with phantoms are routinely compared with those obtained in the actual patient irradiations.

J.J. Broerse

Calculation and measurement of absorbed dose and neutron spectra inside a biological object with reference to fast neutron radiotherapy

The aim of neutron dosimetry for radiotherapeutic applications is to determine the physical parameters which are necessary to assess the biological response of both the tumour to be destroyed and the surrounding normal tissues. The set of physical quantities should include the fast neutron absorbed dose, the absorbed dose of contaminating gamma-radiation and radiation quality. The assessment of the radiation quality, which can be related to the neutron energy spectrum or the LET spectrum, is essential in view of the dependence of RBE on neutron energy

In the present report the collimator design and the dosimetry techniques employed for 15 MeV neutron irradiations are described.

The 15 MeV neutron generator employed is a modified Texas Nuclear model 9909S accelerator, with a separate oil insulated power supply. After installation the neutron generator showed serious design and construction deficiencies. The machine was rebuilt; essential parts such as the ion source and the Einzel lens were replaced by ORTEC equipment, the acceleration tube was disconnected and reconstructed according to appropriate sealing procedures. A total neutron yield of $6 \cdot 10^{11}$ neutrons/sec produced by the D-T reaction is obtained at operation conditions of 270 kV and 6 mA deuteron beam current.

In previous studies it has been found that steel is the most effective shielding material per unit thickness for a primary beam of 15 MeV neutrons. This material has to be followed by hydrogenous layers in order to attenuate the secondary neutrons of lower energies and a subsequent layer of high density material to attenuate the gamma-radiation produced by interaction processes of the neutrons with the shielding materials. Due to the limited yield of the neutron generator employed, the actual thickness of the clinical collimator had to be restricted to a total thickness of 40 cm. On the basis of the previous attenuation studies it can be estimated that for this particular shield, consisting of 5 cm polyethylene

close to the target, followed by 15 cm steel and four alternate layers of 5 cm polyethylene and 5 cm steel, the relative transmission of the total dose amounts to 4%. The polyethylene layer close to the target was introduced in order to avoid high-level activity induced in this first layer. The actual collimator employed for our patient irradiations is shown in figure 1. The last steel layer was covered with a 1 cm thick lucite plate to decrease the low energy neutron contribution, whilst a 3 mm thick teflon layer was positioned at the patient entrance field in order to obtain a maximum skin-sparing effect.

The tissue-equivalent ionization chambers employed in previous experiments are too large to be applied for in-phantom dosimetry. Consequently two chambers of smaller dimensions have been constructed, namely a spherical ionization chamber and a pencil-type ionization chamber. The spherical ionization chamber, with an outer diameter of 23 mm and a wall of tissue-equivalent plastic of 3 mm thickness, was constructed on a modified stem of an existing instrument.

The gamma contamination of the neutron field was estimated by comparing the response of tissue-equivalent ionization chambers with that of a carbon dioxide-flushed teflon chamber and an argon-flushed magnox chamber. The latter type of chambers have a small neutron sensitivity, which is indicated by the coefficient k . This coefficient k is dependent on the neutron energy. On the basis of the kerma values for carbon, carbon dioxide and soft tissue, it was calculated that for the carbon dioxide flushed teflon chamber the coefficient will be equal to 0.32 for 15 MeV neutrons. For the argon-flushed magnox chamber the coefficient k was estimated to be equal to 0.14 for 15 MeV neutrons.

The depth-dose studies were carried out in two different phantoms of the upper part of the human body, namely a phantom made of a fluid rubber compound and an elliptical polyethylene cylinder filled with tissue-equivalent liquid. Several iso-dose curves have been determined for various types of phantoms at a TSD of 45 cm and a field size of 6 x 8 cm, using the pencil type tissue-equivalent ionization chamber.

For each patient, data of the tumour size and position relevant to the surrounding tissue were generally obtained from X-ray transversal planigrams; on the basis of this information a depth-dose curve for the patient was prepared

using the relevant curve from the fluid rubber phantom. An example of the method used for plotting such a treatment planning curve is shown in figure 2. For the majority of the patients the neutron irradiations were carried out bilaterally - primarily to reduce the dose to the skin but also to provide a better dose distribution over the tumour.

In order to evaluate the actual dose received by the tumour, sulphur activation detectors were employed, which were attached onto the skin of the patient at the exit position of the beam. A summary of the patient monitoring results can be found elsewhere (Broerse, Broers-Challiss and Maruyama, 1972).

The project will be continued with measurements for additional field sizes and improved collimator characteristics. The total absorbed dose at various sites will be measured using tissue-equivalent ionization chambers, whilst an estimate of the gamma-contamination will be derived using neutron insensitive GM counters. In addition the biologically effective dose at various positions in the phantom will be determined using an established line of cultured cells of human kidney origin as a biological dosimeter.

LIST OF PUBLICATIONS CONTRACT No. 113-72-1 BIOC

- Broerse, J.J., Broers-Challiss, J.E. and Maruyama, T., Dosimetry of D-T neutrons for radiotherapeutic applications. In: Proc. 1st Symp. on Neutron Dosimetry in Biology and Medicine, Munich, Germany (1971). Commission of the European Communities, Euratom, Luxembourg, EUR 4810 d-f-e, pp. 627-647 (1972).
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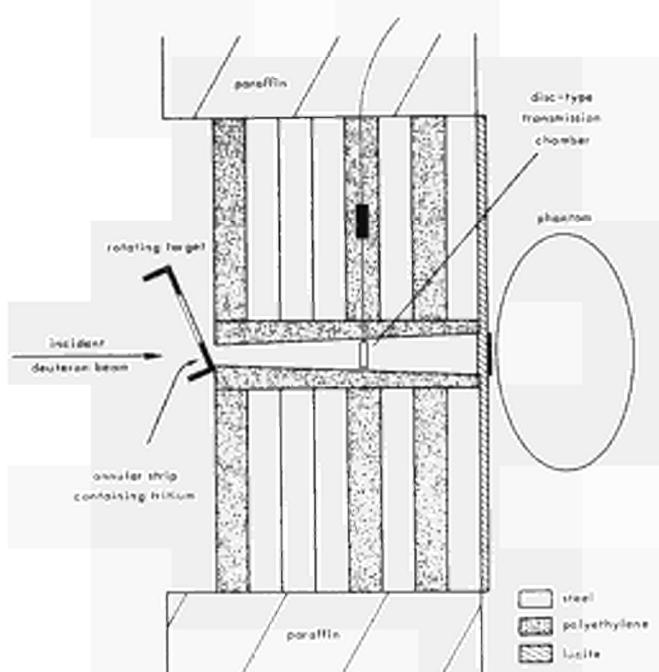


Figure 1. Collimator with a horizontal beam, consisting of alternate layers of steel and polyethylene, employed for patient irradiations with 15 MeV neutrons produced by the D-T reaction. The last steel layer was covered with a 1 cm thick lucite layer, whilst a 3 mm thick teflon layer was positioned at the patient entrance field.

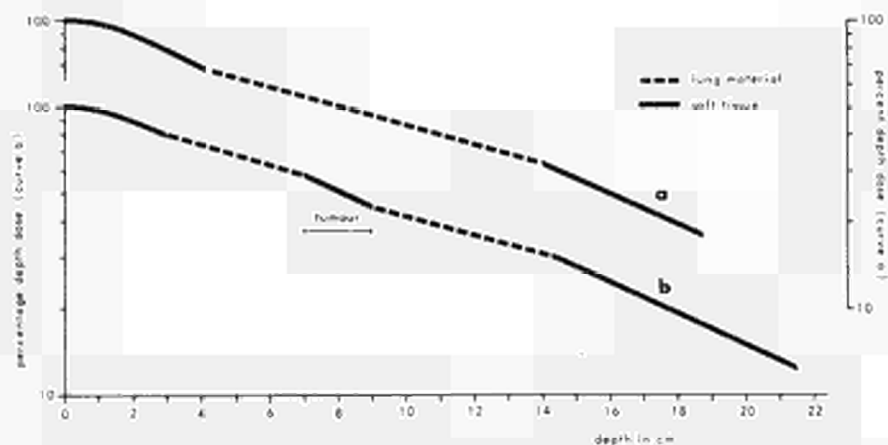


Figure 2. Central axis depth-dose curve for a tissue-equivalent phantom containing lung material (curve a) and the curve applied for the treatment of a patient (curve b).

Contractor: International Commission on Radiation Units and Measurements

Contract No.: 113-72-1BIOC

Head of research team(s): H.O. Wyckoff

General subject of Contract: ICRU Neutron Dosimetry Intercomparison Project

The ICRU Intercomparison Project has to do with the comparison of measurements obtained by a dozen senior investigators representing a dozen different laboratories. Narrow bands of neutron energies as well as neutrons from a californium source are available for the intercomparison. Measurements are to be in terms of the tissue kerma rate in free air and the tissue dose rate at depth in a liquid phantom. The program calls for a set of three laboratory intercomparisons at a time. It is expected that the comparisons themselves will be completed during the calendar year 1973. At the end of the reporting period (January 31, 1973) no comparisons had yet been done, although a schedule for them had been agreed to and the physical facilities completed and tested.

KOORDINIERUNGSTÄTIGKEIT

COORDINATION

ACTIVITES DE COORDINATION

VI. COORDINATION

1. The research subjects approved by the various Council bodies and making up the Biology - Health Protection Programme were determined in close collaboration fostered between the representatives of the Commission and the national experts. In this way it was possible to arrive at a broad definition of the projects to be embarked upon and to draw up the basic data of the Commission's programme. In fact, the implementation of the provisions of the Treaty of Rome in this area had served to demonstrate from the very start that the coordinating role of the Commission was an absolute prerequisite for the formulation and execution of Community objectives.

2. This coordination task was to assume many different guises and to evolve only gradually. In particular, a distinction had to be drawn between the following:
 - 2.1. Preliminary coordination involved in the drawing-up, implementation and execution of the Commission programme, i.e., coordination which relies on outside contributions for the realization of a joint project.

 - 2.2. Coordination which could be described as external, its purpose being to enable national laboratories or individual research workers to benefit from the results of this joint action mainly through the exchange of information, the creation of European groups of research workers or laboratories and the exchange of research workers.

- 2.3. One particular aspect of coordination connected with the Health Protection Directorate's own activities in relation to the Euratom directives on basic standards.

Admittedly, after several years of practical experience it has emerged that these various types of coordination are closely linked and interrelated. Nevertheless, there are a number of aspects of these activities which need to be examined in greater detail.

- 3.1. The Commission programme is executed primarily on a contractual basis.

The first coordinating stage has consisted in defining the various programme objectives. This has been the task of the "programme groups", made up either of individual experts (the former Advisory Committee on Biology) or of national experts working within an institutionalized group (ad hoc group of the Advisory Committee on Nuclear Research) which drew up the basic data for the Biology - Health Protection Programme.

The second coordination stage has consisted and still consists in selecting contractors on the basis of the research subjects which the latter propose to study. The two characteristic features of this stage are as follows:

- (a) Assessment of projects and research findings of contractors in relation to the general programme (at present the Advisory Committee on Programme Management, an institutionalized body, is responsible for carrying out this task).
- (b) Coordination of research carried out by different contractors working in the same field. This is

achieved by the grouping together of contracts so as to ensure continual contact between a wide variety of national laboratories and the Commission on the basis of continuing and meaningful cooperation fostered by the Management Committees which steer all association contracts (and which, in the case of the CEA Association on levels of contamination of the food chain and of the ITAL Association are advised by an international group of scientific consultants), as well as by Study Groups, to which we will revert in a moment.

A further stage in the process of coordination was introduced when the need became apparent for continuous supervision of the progress being made in the various fields of research, so as to ensure that this research was of the highest possible quality employing the most modern techniques. Accordingly, the Commission decided that an effective method would be to set up Study Groups consultative to the Commission, the latter being responsible for appointing the members of the groups.

Up to now these Study Groups have examined the "state of the art" in the various sectors of the general programme and have attempted to define the methods of research to be adopted in each sector. The basic, if not sole, task of the groups is to arrive at an assessment, based on free, informal discussions, of the state of progress in each of the research sectors and to put forward recommendations for appropriate guidelines.

As far as the Commission is concerned, the primary role of the Study Groups has been, and must continue to be, to serve as scientific forums bringing together, for each

programme sector or objective, the parties under contract to the Commission, the Commission itself and also independent experts.

The Health Protection Directorate also includes groups of experts whose activities have the effect both of coordinating and stimulating efforts towards practical measures of radiation protection (up-dating the Euratom directives) and the furthering of research on radiation protection. These groups are, in particular, the "basic standards committee" mentioned in article 31 of the Euratom Treaty and the group of experts for "personnel dosimetry - health protection", to which a number of institutes and laboratories from all the Community countries is attached for the implementation of comparative programmes.

Attached is a list of the Study Groups which met in 1972*.

- 3.2. The Study Groups and their dominant role in the work of coordination must not be allowed to obscure the fact that the Commission has implemented a wide variety of measures designed to benefit the scientific community. By way of example, brief reference could again be made to the so-called group contracts through which it has been possible to bring together within the same legal framework many different national laboratories and consequently to create contacts between research workers from these laboratories and promote an exchange of information in each of the specific areas of research. This applies in the case of the Universities of Brussels and Pisa, the EORTC, in

* This annex is divided into two parts: (A) Study Group meetings during 1972; (B) Meetings organized or co-sponsored by the Commission.

the field of dosimetry, etc. By introducing and continuing to promote these measures the Commission has assumed the characteristic role of catalyst, a role which it must continue to play. Other similar measures take the form not only of the exchange of oral, informal information but also the publication of reports (EURATOM reports, newsletters) and the exchange of research workers.

Furthermore, in order to encourage even closer cooperation, the Commission has, within the limits of its financial resources, taken the initiative in organizing or actively supporting scientific meetings of the most significant kind. Suffice it to mention, in this connection, the symposia on microdosimetry organized every two years.

Lastly, by awarding fellowships and setting up training courses for young research workers under the terms of a Convention, the Commission has endeavoured to prepare the Community's future research workers for closer European cooperation, in the hope that they will continue to act in this spirit after they have established themselves in the research field.

A. Meetings of
Study Groups in 1972

Study Group "Dosimetry"

Frankfurt, 20-21 March 1972,
22 participants from 6 countries and the Commission.

Principal subject:

Energy transfer in biological material and in model substances.

Meeting of the technical experts on "Personnel Dosimetry"

Luxembourg, 28 April 1972,
18 participants from 5 countries and the Commission.

Principal subjects:

Exchange of views on the results of the programme executed in 1971
on the intercomparison of neutron and photon dosimeters.

Discussion of the state of work in progress concerning the programme
on intercomparison of neutron sources.

Discussion on the harmonisation of thermoluminescence dosimeters
in the member states of the Community.

Extension of the programme on intercomparison on ambient moni-
toring and accidental dosimetry; measurements of response curves
from different dosimeters.

Study Group "Thorotrast"

Heidelberg, 16 June 1972,
15 participants from Germany and the Commission.

Principal subjects:

State of the Thorotrast programme.

Examination of patients with Thorotrast burden using the mobile
monitor station for those unable to be moved.

Report on the Portuguese and the North-american study on Thorotrast.

First results from the follow-up study.

Hydronephrosis due to paravenous Thorotrast injection.
Thorotrast excitation and radiation effect.
Determination of morphological alterations in Thorotrast conglomerates by electronic picture analysis.

Study Group "Thermoluminescence Dosimeters"

Braunschweig, 16 June 1972,
17 participants from 6 countries and the Commission.

Principal subject:
Preparation of technical recommendations for the use of thermoluminescence in personnel dosimetry.

Study Group "Primary Effects of Radiation on Nucleic Acids"

Brussels, 19-20 June 1972,
18 participants from 7 countries and the Commission.

Principal subject:
Studies of radical formation, of their properties and their behaviour in nucleic acids in solid and liquid state.

Study Group "Radiation Induction of Virus-Mediated Cancers"

Brussels, 14 September 1972,
4 participants from the Netherlands and the Commission.

Principal subject:
The present state of work on the involvement of viruses in malignancy and possible models of radiation-induction of the malignant state connected with these.

Study Group "Thermoluminescence Dosimeters"

Luxembourg, 25 September 1972,
17 participants from 6 countries and the Commission.

Principal subject:
Preparation of technical recommendations for the use of thermoluminescence in personnel dosimetry.

Study Group "Dosimetry"

Toulouse, 9-10 October 1972,
22 participants from 6 countries and the Commission.

Principal subject:

Research in dosimetry related to

- the evaluation of the biological effectiveness of different types of radiations,
- energy transfer in biological material and in model substances,
- radiation effect in condensed matter and its application.

Study Group "Genetic Effects of Radiations"

Brussels, 24-25 October 1972,
27 participants from 7 countries and the Commission.

Principal subjects:

Review of a wide range of work including

- the dose-effect relation for non-disjunction,
- effects of caffeine on mutagenic processes,
- chromosomal changes due to irradiation in utero in the mouse,
- reactivation by irradiation and its relation to mutagenic processes,
- molecular spectra of induced mutations in yeast and bacteriophage,
- oncoviruses: structures, irradiation, mechanics of action,
- enzymes, mutants and processes involved in repair of radiation damage and mutagenesis in bacteria, yeast, Drosophila and mammalian cells,
- radiosensitivity of hybrid cells.

Study Group "Identification of Irradiated Food"

Luxembourg, 24-25 October 1972,
14 participants from 3 countries and the Commission.

Principal subjects:

Research concerning physical, chemical and biological changes in foods irradiated for preservation.

Research to establish control techniques and practices adequate for the inspection of food after irradiation to ensure compliance with the public health regulations.

Study Group "Pollution Levels in the Environment"

Rome, 25 October 1972,

9 participants from 4 countries and the Commission.

Principal subjects:

The ten-yearly balance of projects in the research group of the Association was presented and discussed with a view to its publication. A programme of new studies was proposed, containing the following points:

- Study of levels of contamination in drinks, especially drinking water.
 - Study of media regarded as factors in food production (soils, fresh water, oceans), taking into consideration regional variability and its influence on pollution transfer.
 - Study of the distribution system for agricultural products regarded as factors in the dilution of contamination.
-

Study Group "Personnel Dosimetry"

Luxembourg, 29 November 1972,

15 participants from 6 countries and the Commission.

Principal subjects:

Report of the contracting parties on the work carried out in 1972.

Discussion of the results obtained from the research in 1972 and on the planning of work for 1973.

Study Group "Thermoluminescence Dosimeters"

Luxembourg, 30 November 1972,

17 participants from 6 countries and the Commission.

Principal subject:

Preparation of technical recommendations for the use of thermoluminescence in personnel dosimetry.

Study Group "Thorotrast"

Heidelberg, 1 December 1972,
12 participants from Germany and the Commission.

Principal subjects:

Registration and examination of patients and of the control group of Thorotrast.

Radiation effects, sensitivity to foreign bodies, dosimetry, statistical evaluation.

Study Group "Chromium"

Wageningen, Netherlands, 14-15 December 1972, .
14 participants from the Netherlands and the Commission.

Principal subject:

Behaviour of Chromium in soils, plants, fresh water and aquatic organisms.

B. Meetings

organized or co-sponsored by

the Commission of the European Communities in 1972

"Marine Contamination" Group

Rovinj Yugoslavia, 8-9 February 1972,
29 participants from 7 countries and the Commission.

Principal subjects:

- Physico-chemical forms of marine microconstituents and their relation to the accumulation of radionuclides.
 - Conventional pollutants studied with radionuclides.
 - The case of the Gulf of Taranto as regards radiocontamination.
-

Symposium on Neutron Dosimetry in Biology and Medicine

Neuherberg/München, 15-19 May 1972.

This Symposium was held in view of the increasing application of neutrons for biological, medical and technical purposes, to evaluate the radiation risk and to assess the physical basis of radiological applications of neutrons.

150 scientists from 18 countries and the Commission had a detailed exchange of views on recent work. The contractual activities of the Commission in neutron dosimetry (contract nr 113-72-1 BIOC) initiated in 1972, received a strong stimulation.

Proceedings, containing 58 papers, are published as Euratom Report nr EUR 4896.

Seminar on the Possibilities of Applying the Effects of Exoelectronic Emissions to Dosimetry

Braunschweig, 12-13 June 1972.

The aim of this seminar was a mutual exchange of information on the present state of research in exoelectron dosimetry and on exoelectronic research in general, to improve contacts between the various disciplines and to determine what direction future research is likely to take in this field.

On the basis of the preparatory work already done, very promising to be applied in health protection, it was the unanimous opinion of the participants that the collaboration should be intensified and the research coordinated from the exoelectron dosimetry point of view.

The greater sensitivity of exoelectron dosimeters promises advantages over methods currently in widespread use, involving the determination of radiation doses absorbed by persons handling radioactive substances from the darkening of photographic film worn on the body.

This meeting was organized in conjunction with the Physikalisch-Technische Bundesanstalt at Braunschweig and 40 experts from 8 countries and the Commission attended this meeting.

Fourth Annual Plenary Meeting of the European Organization for Research on the Treatment of Cancer (E.O.R.T.C.)

Paris, 23 June 1972,
80 participants from 10 countries and the Commission.

Principal subjects:

- The viral etiology of cancer was evaluated using the human mammary tumor virus, the human sarcoma virus and leukemia viruses as paradigmatic models of recent interest.
- The problem of the use of hormones in the treatment of certain types of cancer, of the combination of cytotoxic and radiomimetic agents to combat neoplasia and the possibility to synchronise cells by cycle specific agents. Since new cytotoxic agents have been developed, part of the discussion was directed to compare new trends in the field in USA and Europe.
- The use of aseptic environments to treat patients with hematopoietic failure secondary to malignant disease, cytotoxic drugs or radiation.

In this context, the Euratom supported E.O.R.T.C.-Gnotobiotic Project Group could report about significant advances.

European Late Effects Project Group (EULEP)

- Committee on carcinogenesis.
- Committee on the study of displasic and dystrophic lesions and of the quantitative and qualitative modifications of the cell.
- Committee on animal standardization.

Mol, Belgium, 9-11 November 1972,
25 participants from 8 countries and the Commission.

Principal subject:

Discussions of new research programmes to be realized by several laboratories of the EULEP Group.

"Mutation Breeding Contact Group"

Rome, 11-14 December 1972,
25 participants from 3 countries and the Commission.

Principal subjects:

- Incompatibility problems.
 - Protein improvement.
 - Disease resistance induction.
-

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